Influenza Vaccination in Community Dwelling Elderly Persons

Bettie Voordouw

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Influenza Vaccination in Community Dwelling Elderly Persons

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You see things;
and you say, “Why?”
But I dream things that never were;
and I say, “Why not?”
(George Bernard Shaw)

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General introduction

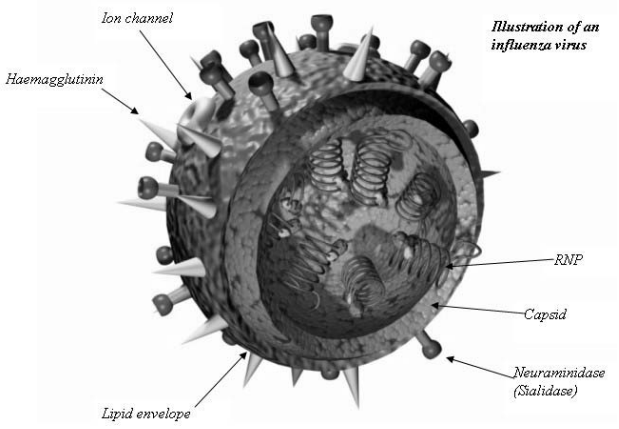
1 Introduction

An influenza epidemic was first described in 1173, although there are reports of influenza as early as 412 BC [1]. Recurrent epidemics and incidental pandemics caused by influenza virus are documented since the last 400 years [1, 2]. These were based upon clinical observation and epidemiology. Although a virus was suspected to be the cause of influenza infections, it was not until 1933 that influenza viruses were first isolated during an influenza epidemic in London [3]. Only recently the virus causing the 1914-1918 pandemic ("Spanish flu") was isolated from conserved lung tissue of an American soldier [4]. The first influenza vaccine was licensed in 1945 [1].

2 The influenza virus

The influenza virus belongs to a family of viruses called Orthomyxoviridae. Three genotypes of influenza virus exist, namely influenza A, influenza B, and influenza C. Type A is commonly associated with epidemics and has many mammalian hosts. Type B infects only humans and causes disease, however, less severe than type A. Type C only infects humans but is thought to be harmless. This strain is genetically and morphologically distinct from the A and B genotypes. Influenza virus particles are pleiomorph, mostly ovoid or spherical, with a diameter of 80-120nm. The capsid of the virus is surrounded by a lipid envelope, attached to which are two types of glycoprotein spikes (haemagglutinin and neuraminidase) and one type of ion channel.

figuur 1. influenza virus



(Picture from: http://www.omedon.co.uk/influenza/influenza)

3 Influenza epidemiology

Today, epidemics are generally caused by influenza A (H3N2 or H1N1) or B viruses. Influenza A/H2N2 subtypes have not been isolated since 1967. Influenza C virus is often isolated during epidemics with either influenza A or B viruses.

Annual influenza epidemics are associated with substantial morbidity and mortality, especially in elderly and in those with underlying diseases [5-8]. During winter periods, up to 30% of elderly may experience acute respiratory infections of which up to 20% are attributed to influenza virus [9-12]. Although viral pneumonia accounts for a higher mortality rate than bacterial pneumonia [2], in elderly mostly secondary bacterial pneumonia associated with influenza (P&I) contributes substantially to excess mortality [5]. In the US, the combined cause of death category ranks pneumonia and influenza as the sixth leading cause, mostly in elderly patients. In 1992, persons aged ≥65 years accounted for 89% of all pneumonia associated with influenza deaths [13]. Based on data from Statistics Netherlands (CBS) it was estimated that during a 22.5 year period more than 2000 people died from influenza each year [8]. Incidence of influenza related mortality increases substantially with increasing age, from 82 per 100,000 in persons aged 60-69 to 280 per 100,000 in persons older than 80 years [8] In the US, similar death rates are reported [13]. For the 2004-2005 season, the CBS calculated an excess of 800 deaths in the Netherlands, despite the relatively mild epidemic activity [14]. Although in healthy adults clinical influenza infections generally have an uncomplicated course, it may have a substantial economic impact, e.g. due to days lost from work [15].

In the Netherlands an epidemic is declared when at least 15 per 10,000 inhabitants report symptoms of influenza like illness (ILI) (i.e., the epidemic threshold) [16]. During the last 10 years, epidemics in the Netherlands were generally of mild to moderate severity. The highest incidence was reached in the 1994-1995 season with 43 reported cases of ILI per 10,000 inhabitants [17]. During the 2000-2001, 2001-2002 and 2002-2003 seasons the epidemic threshold was not reached. In the 1994-1995, 1995-1996 and 2004-2005 seasons there was a moderate match between circulating strains and virus strains [14, 17, 18]. In the 1997-1998 season there was a mismatch for A/H3N2 [19] and in 2003-2004 there was a mismatch for the B strain, but a good match for A/H3N2 [20]. The other seasons were characterised by a good match between circulating strains and vaccine strains [21].

4 Clinical symptoms and complications following influenza infections

Clinical manifestations of influenza infection include abrupt onset of fever, myalgia, sore throat, non-productive cough, headache and malaise. In most cases an influenza infection is presented as influenza like illness.

For the purpose of registration of epidemic activity different definitions are used to reach uniform recording of influenza like infection cases. In the Netherlands, the definition of the sentinel station is used [16]. Fulfilment of this definition requires an acute onset of clinical disease, with a maximum duration of the prodromal stage of 3-4 days (including pre-existing respiratory infection at a non-sickening level). Furthermore, the infection should be accompanied by an increase in rectal temperature to at least 38°C and at least one of the following symptoms: cough, coryza, sore throat, frontal headache, retrosternal pain, myalgia. The internationally used ICHPPC-2 definition [22] is somewhat different. This definition also assumes a sudden onset with cough, shivering, fever, malaise, headache, myalgia, sore throat or rhinitis, but also contact with influenza. Diagnosis should furthermore fulfil at least one of the following 3 criteria: a proven infection by virus culture or serology, or a declared influenza epidemic plus at least 4 of the above mentioned symptoms or no declared epidemic with at least 6 of the above mentioned symptoms.

Most frequently reported complications associated with influenza are primary influenza viral pneumonia and secondary bacterial pneumonia. Influenza viral pneumonia occurs predominantly among persons with cardiovascular disease (especially rheumatic disease with mitral stenosis). Secondary bacterial pneumonia occurs most often in elderly who have chronic pulmonary, cardiac and metabolic or other disease [2, 23]. Other pulmonary complications of influenza are croup and exacerbations of chronic obstructive pulmonary disease, both mainly afflicting high risk children and adults [2, 23].

The non-pulmonary complications include myositis (mostly children), cardiac complications (myocarditis and pericarditis), toxic shock syndrome (healthy children and adults) central nervous system complications (Guillain-Barré syndrome, transverse myelitis, encephalitis) and Reye syndrome (almost exclusively in children between 2-16 years with a mortality rate of 10-40%) [2, 23].

5 Influenza vaccination

The main objective of influenza vaccination in elderly is reducing the number of deaths caused by influenza infections. Prevention against influenza is possible through active immunisation with influenza vaccine. The composition of this vaccine

is determined yearly at a WHO consensus meeting, and is based upon the expected circulating strains.

Each vaccine used in annual influenza vaccination programs combines the antigenic components of 2 influenza A strains (H3N2 en H1N1) and 1 influenza B-strain, the so-called trivalent influenza vaccines. In adults and elderly, vaccination against influenza is recommended as a single injection containing of each of the three components of the trivalent vaccine (i.e., the A/H3N2, A/H1N1 and B strain). Antibody development may start as early as one week following vaccination [24], but peak antibody responses are observed after approximately 4 weeks [25] and these may last for at least 24 weeks in healthy elderly [26].

A main limitation for the possible effectiveness of influenza vaccination programs is that the composition is determined by the circulating strain(s) of the previous year.

6 Influenza vaccination program in the Netherlands

Since 1997 the Dutch government recommends influenza vaccination of all elderly over 65 years of age. This is a centralised program executed by General Practitioners (GPs). The GPs invite the patients from their practice fulfilling the predefined inclusion criteria. During national campaigns in October and November, most individuals who were invited are vaccinated during mass vaccination days at the GP practice. For the vaccinee, the immunisation is free of charge. The GPs are reimbursed by the government for each patient vaccinated in the campaign through the General Law on Special Medical Expenses (AWBZ).

7 Influenza vaccines

Influenza vaccines currently on the market in the EU are prepared as either split virus vaccines or subunit vaccines. The split virus vaccine contains inactivated purified split virion. The subunit vaccine contains purified envelope antigens through selective solubilisation of the envelope. Subunit vaccines are suggested to be less reactogenic. On the other hand, however, a comparative study between a subunit vaccine and split vaccine indicated better immunogenicity of the split vaccine [27].

7.1 Immune response following influenza vaccination

To prevent influenza infection, adequate quantities of anti-haemagglutinin (HI) antibodies must be present (i.e. the humoral response) in the vaccinated person. Antibody to the virion surface neuramidase (anti-NA) and activated cellular immunity through cytotoxic T cell activation can aid in reducing the severity and duration of infection [28].

The humoral immune response against influenza virus infection is predominated by immunoglobulin G (IgG) antibodies. Intramuscularly administered inactivated influenza vaccines stimulate primarily IgG antibodies and after intramuscular immunisation most recipients develop these serum IgG antibodies. However, in the upper respiratory tract immunoglobulin A (IgA) produced locally by submucosal lymphocytes may predominate [28, 29]. The rate of locally produced IgA antibodies in nasal washings is variable (10-30% of young adults, <10% in elderly) and (when present) of short duration [30, 31].

Contrary to the humoral immunity which is strain-specific, cell-mediated immunity following vaccination, conferred by T-helper cell and cytotoxic T lymphocytes, is cross-reactive with many other strains of influenza A and B [31]. Thus cell-mediated immunity stimulated by vaccination will, in principle, offer protection against many influenza viral strains. Ageing affects B cell function [32] and T cell function [33]. Since for the production of antigen specific antibodies, B cells are dependent upon T cell helper function, T cell dysfunction in elderly may lead to a reduction in humoral responses. This is also occurring in healthy elderly. Improvement of the cellular immunity is thus important to improve the humoral immune response to vaccination. Different strategies are investigated to achieve this goal. For the regular inactivated vaccines these include annual revaccination [34-37], an increase in the dose [38-40] or the recommendation of a booster dose [26, 41-44]. Other developments include live attenuated virus vaccines [42, 45-47], alternative routes of administration [48, 49], and the use of adjuvants or other immunomodulators [50-53]. Only annual revaccination is generally recommended. None of the other strategies have yet proven to be successful.

7.2 Immunogenicity and clinical protection following influenza vaccination

In immunogenicity trials seroresponse following vaccination is usually measured with the haemagglutination inhibition (HI) assay as proxy for clinical protective efficacy. For the HI assay, three parameters are regarded.

1. The proportion of subjects exceeding a post-vaccination titre of 40 (seroprotection rate).

2. The mean geometric increase (also called mean fold increase, MFI), i.e. the geometric mean of the quotients of post- and pre-vaccination titres.
3. The combined proportion of previously seronegative subjects (prevaccination HI titre<10) exceeding a post-vaccination titre of 40 and that of previously seropositive subjects (prevaccination titre ≥10) with a ≥4-fold increase in GMT (response rate).

The parameters are described in the CHMP Note for Guidance on harmonisation of requirements for influenza vaccines [54].

The primary endpoint in clinical studies depends upon the target population. For elderly persons, the primary endpoint is usually hospitalisation for (influenza associated) pneumonia or other respiratory diseases and mortality. Clinically defined influenza like illness and serologically or virologically confirmed influenza infection is a less often used endpoint in elderly, especially in observational studies.

In experimental randomised, controlled trials, the clinical protection achieved in the vaccinated group compared to the non-vaccinated group is expressed as vaccine efficacy. In observational cohort or case control studies clinical protection achieved in the vaccinated population compared to the non-vaccinated population is expressed as effectiveness of vaccination.

In a randomised, placebo controlled trial, performed in healthy elderly aged 60 years and older seroprotection was found in 43-68% of the vaccinated group [55]. In the same study population, vaccination also halved the risk of developing serologically defined influenza, i.e., vaccine efficacy was 50% ($CI_{95\%}$: 29-67%). The corresponding incidences of serologically defined influenza were 9% in the placebo group and 4% in the vaccine group [56]. But in the vaccinated group, this incidence was only 0.9% in patients with a vaccination history, whereas it was 5.1% in those not previously vaccinated.

Effectiveness of vaccination depends upon other population factors and environmental factors in addition to the intrinsic efficacy of the vaccine. The population factors include age, health status and living conditions of the vaccinee.

Meta-analyses have reported a benefit of vaccination in a variety of outcomes [57-59]. In a meta-analysis from cohort studies in institutionalised elderly, the pooled risk reductions were substantial: 56% for respiratory illness, 53% for pneumonia, 50% for hospitalisation and 68% for mortality [58]. For community dwelling elderly the pooled estimates appeared slightly less favourable, with a 35% risk reduction for influenza like illness, 47% for hospitalisation for pneumonia and influenza and 50% for all cause mortality [57]. In a meta-analysis addressing single vs. multiple vaccinations, the pooled protection rate difference for the outcome confirmed influenza was close to 0, indicating no difference between single and multiple vaccination [60]. In this meta-analysis only 2 out of the 4 included studies were in elderly.

The environmental factors include antigenic match of vaccine strain and circulating strain and vaccination coverage. The annual changes in the existing A and B strains (i.e. “antigenic drift”), may result in variable effectiveness of vaccination, even when adequate serological responses would be achieved. Systematic surveillance during the influenza season has confirmed widespread infection among older patients, both in the community and in institutions [61]. Increased vaccination coverage might positively influence vaccine effectiveness through indirect protection (herd-immunity), although this may be better achievable for institutionalised persons than for elderly living in the community. Herd immunity is associated with a lower dependency on the antigenic match between vaccine and virus [62-64]. For example, in a study in a nursing home outbreak, overall influenza vaccination failed to protect against morbidity or mortality outcomes, but not in those individuals vaccinated annually during the last 5 years [63]. A surveillance in 58 nursing homes during the same year showed that outbreak frequencies were inversely related to vaccination coverage and the number of beds [64]. To increase or sustain clinical protection, annual revaccination and/or achieving a high vaccination coverage might therefor be instrumental.

8 Licensure of influenza vaccines

Influenza vaccines submitted for first time use within the European Union, are licensed following assessment of their quality, efficacy and safety. Because in general, influenza vaccine composition changes each year, following recommendations of WHO, vaccines need to be relicensed on an annual basis. For annual relicensure purposes and in accordance with the European Committee of Human Medicinal Products (CHMP) Note for Guidance (CHMP/BWP/214/96) marketing authorization holders of influenza vaccines perform small scale immunogenicity trials in a selected population of healthy adults (age 18-60 years) and elderly (age >60 years) [54].

Such trials are evaluated using predefined criteria described in the Note for Guidance. For adults, postvaccination seroprotection rate should be achieved in at least 70% and response rate in at least 40%. For elderly the corresponding frequencies should exceed 60% and 30% respectively. A current limitation of the guideline is, however, that it does not specify criteria for children or indivuals with underlying chronic disease for which influenza vaccination is recommended. Therefor, the clinical relevance of these criteria is frequently questioned.

9 Aim and outline of this thesis

The first aim of this thesis was to get a better insight into the benefit of annual influenza vaccination in community dwelling elderly immunised as part of the national influenza vaccination program. For this purpose 4 epidemiological studies were conducted in a population of community dwelling elderly which was selected and characterised from the IPCI database. These studies are summarised in chapters 2-5. In this population the impact of annual vaccination on mortality and lower respiratory infections was studied.

A second aim of this thesis was to study the ability of clinical trials performed for annual relicensure of influenza vaccines to detect (differences in) the vaccines' immunogenicity of that particular year (chapter 6).

A final aim was to investigate the impact of vaccination on immunological and clinical outcomes. For this purpose we performed a meta-analyses described in a review in chapter 7.

References

1. Nicholson, K.G., *Human Influenza*, in *Textbook of influenza*, K.G. Nicholson, R.G. Webster, and H. A.J., Editors. 1998, reprint 2000, Blackwell sciences ltd.: London. p. Chapter 1 & 2, pages 3-25.
2. Mandell, G., *Orthomyxoviridae. Influenzavirus*. Mandell, Douglas, Bennett.'s principles and practice of infectious diseases., 1995(4th edition): p. Chapter141, pages 1546-67.
3. Smith, W., C. Andrews, and L. PP, *A virus obtained from influenza patients*. Lancet, 1933. 2: p. 66.
4. Taubenberger, J.K., et al., *Initial genetic characterization of the 1918 "Spanish" influenza virus*. Science, 1997. 275(5307): p. 1793-6.
5. Barker, W.H. and J.P. Mullooly, *Pneumonia and influenza deaths during epidemics: implications for prevention*. Arch Intern Med, 1982. 142(1): p. 85-9.
6. Thompson, W.W., et al., *Mortality associated with influenza and respiratory syncytial virus in the United States*. JAMA, 2003. 289(2): p. 179-86.
7. Fleming, D.M., *The contribution of influenza to combined acute respiratory infections, hospital admissions, and deaths in winter*. Commun Dis Public Health, 2000. 3(1): p. 32-8.
8. Sprenger, M.J., et al., *Impact of influenza on mortality in relation to age and underlying disease, 1967-1989*. Int J Epidemiol, 1993. 22(2): p. 334-40.
9. Falsey, A.R., et al., *Acute respiratory tract infection in daycare centers for older persons*. J Am Geriatr Soc, 1995. 43(1): p. 30-6.
10. Glathe, H. and W. Lange, *Influenza vaccination in older patients. Immunogenicity, epidemiology and available agents*. Drugs Aging, 1995. 6(5): p. 368-87.
11. Nicholson, K.G., et al., *Acute viral infections of upper respiratory tract in elderly people living in the community: comparative, prospective, population based study of disease burden*. BMJ, 1997. 315(7115): p. 1060-4.
12. Walsh, E.E., C. Cox, and A.R. Falsey, *Clinical features of influenza A virus infection in older hospitalized persons*. J Am Geriatr Soc, 2002. 50(9): p. 1498-503.
13. *From the Centers for Disease Control and Prevention. Pneumonia and influenza death rates--United States, 1979-1994*. JAMA, 1995. 274(7): p. 532.
14. Nieuwsbrief influenza surveillance 2004/2005, 2005. Jaargang 13(nr.9): p. (http://www.virology.nl).
15. Nichol, K.L., et al., *The effectiveness of vaccination against influenza in healthy, working adults*. N Engl J Med, 1995. 333(14): p. 889-93.
16. (NIVEL). C*ontinue morbiditeits registratie peilstations Nederland 1988/89*. 1991, Nivel: Utrecht.
17. Claas, E.C., et al., *[Influenza in the 1994/95 season; composition of vaccine for the 1995/96 season]*. Ned Tijdschr Geneeskd, 1995. 139(42): p. 2154-8.
18. Claas, E.C., et al., *[Influenza in the 1995/'96 season; vaccine composition for the 1996/'97 season]*. Ned Tijdschr Geneeskd, 1996. 140(41): p. 2047-50.
19. de Jong, J.C., et al., *Mismatch between the 1997/1998 influenza vaccine and the major epidemic A(H3N2) virus strain as the cause of an inadequate vaccine-induced antibody response to this strain in the elderly*. J Med Virol, 2000. 61: 94-9.
20. Rimmelzwaan, G., et al., *The 2003/2004 influenza season in the Netherlands with a limited epidemic of the virus variant A/Fujian, and the vaccine composition for the 2004/2005 season*. NTvG, 2004. 48(40): p. 1984-8.
21. Voordouw, A.C.G., et al., *Annual revaccination against influenza and mortality risk in community dwelling elderly persons*. JAMA, 2004. 292(17): p. 2089-95.
22. WONCA, C.C.o., *ICHPPC-2 defined. Inclusion criteria for the use of the rubrics of the International Classification of Health Problems in Primary Care*. 1983, Oxford: Oxford University Press.

23. Nicholson, K.G., *Human Influenza*, in *Textbook of influenza*, K.G. Nicholson, R.G. Webster, and H. A.J., Editors. 1998, reprint 2000, Blackwell sciences ltd.: London. p. 233-244.
24. Cox, R.J., et al., *An early humoral immune response in peripheral blood following parenteral inactivated influenza vaccination*. Vaccine, 1994. 12(11): p. 993-9.
25. Rastogi, S., et al., *Time to peak serum antibody response to influenza vaccine*. Clin Diagn Lab Immunol, 1995. 2(1): p. 120-1.
26. Buxton, J.A., et al., *Influenza revaccination of elderly travellers: antibody response to single influenza vaccination and revaccination at 12 weeks*. J Infect Dis, 2001. 184(2): p. 188-91.
27. Zei, T., M. Neri, and A.M. Iorio, *Immunogenicity of trivalent subunit and split influenza vaccines (1989- 90 winter season) in volunteers of different groups of age*. Vaccine, 1991. 9(9): p. 613-7.
28. Couch, R.B., *Advances in influenza virus vaccine research*. Ann N Y Acad Sci, 1993. 685: p. 803-12.
29. Kuno-Sakai, H., et al., *Developments in mucosal influenza virus vaccines*. Vaccine, 1994. 12(14): p. 1303-10.
30. Clancy, R.L., *Mucosal vaccines for the prevention of influenza*. Drugs, 1995. 50(4): p. 587-94.
31. McElhaney, J.E., et al., *Split-virus influenza vaccines: do they provide adequate immunity in the elderly?* J Gerontol, 1994. 49(2): p. M37-43.
32. Powers, D.C. and R.B. Belshe, *Effect of age on cytotoxic T lymphocyte memory as well as serum and local antibody responses elicited by inactivated influenza virus vaccine*. J Infect Dis, 1993. 167(3): p. 584-92.
33. McElhaney, J.E., et al., *Antibody response to whole-virus and split-virus influenza vaccines in successful ageing*. Vaccine, 1993. 11(10): p. 1055-60.
34. Gardner, E.M., et al., *Characterization of antibody responses to annual influenza vaccination over four years in a healthy elderly population*. Vaccine, 2001. 19(32): p. 4610-7.
35. de Bruijn, I.A., et al., *Annually repeated influenza vaccination improves humoral responses to several influenza virus strains in healthy elderly*. Vaccine, 1997. 15(12-13): p. 1323-9.
36. Beyer, W.E., et al., *Effects of repeated annual influenza vaccination on vaccine sero- response in young and elderly adults*. Vaccine, 1996. 14(14): p. 1331-9.
37. McElhaney, J.E., et al., *The effect of influenza vaccination on IL2 production in healthy elderly: implications for current vaccination practices*. J Gerontol, 1992. 47(1): p. M3-8.
38. Remarque, E.J., et al., *Improvement of the immunoglobulin subclass response to influenza vaccine in elderly nursing-home residents by the use of high-dose vaccines*. Vaccine, 1993. 11(6): p. 649-54.
39. Palache, A.M., et al., *Antibody response after influenza immunization with various vaccine doses: a double-blind, placebo-controlled, multi-centre, dose-response study in elderly nursing-home residents and young volunteers*. Vaccine, 1993. 11(1): p. 3-9.
40. Palache, A.M., et al., *Influenza vaccines: the effect of vaccine dose on antibody response in primed populations during the ongoing interpandemic period. A review of the literature*. Vaccine, 1993. 11(9): p. 892-908.
41. Gross, P.A., et al., *Immunization of elderly people with two doses of influenza vaccine*. J Clin Microbiol, 1987. 25(9): p. 1763-5.
42. Powers, D.C., et al., *In elderly persons live attenuated influenza A virus vaccines do not offer an advantage over inactivated virus vaccine in inducing serum or secretory antibodies or local immunologic memory*. J Clin Microbiol, 1991. 29(3): p. 498-505.
43. Wongsurakiat, P., et al., *Acute Respiratory Illness in Patients With COPD and the Effectiveness of Influenza Vaccination: A Randomized Controlled Study*. Chest, 2004. 125(6): p. 2011-2020.

44. Roos-Van Eijndhoven, D.G., et al., *Randomized controlled trial of seroresponses to double dose and booster influenza vaccination in frail elderly subjects*. J Med Virol, 2001. 63(4): p. 293-8.
45. Gorse, G.J., et al., *Increased anti-influenza A virus cytotoxic T cell activity following vaccination of the chronically ill elderly with live attenuated or inactivated influenza virus vaccine*. J Infect Dis, 1995. 172(1): p. 1-10.
46. Gorse, G.J., et al., *Influenza virus vaccination of patients with chronic lung disease*. Chest, 1997. 112(5): p. 1221-33.
47. Gorse, G.J., et al., *Immunity to influenza in older adults with chronic obstructive pulmonary disease*. J Infect Dis, 2004. 190(1): p. 11-9.
48. Dean, H. and C. D, *Epidermal powder immunization against influenza*. Vaccine, 2004. 23: p. 681-6.
49. Muszkat, M., et al., *Local and systemic immune response in nursing-home elderly following intranasal or intramuscular immunization with inactivated influenza vaccine*. Vaccine, 2003. 21(11-12): p. 1180-6.
50. Squarcione, S., et al., *Comparison of the reactogenicity and immunogenicity of a split and a subunit-adjuvanted influenza vaccine in elderly subjects*. Vaccine, 2003. 21(11-12): p. 1268-74.
51. Frech, S., et al., *Improved immune responses to influenza vaccination in the elderly using an immunostimulant patch*. Vaccine, 2005. 23: p. 946-50.
52. Ben-Yehuda, A., et al., *The influence of sequential annual vaccination and of DHEA administration on the efficacy of the immune response to influenza vaccine in the elderly*. Mech Ageing Dev, 1998. 102(2-3): p. 299-306.
53. Katz, J.M., et al., *Immunity to influenza: the challenges of protecting an aging population*. Immunol Res, 2004. 29(1-3): p. 113-24.
54. *Committee for Proprietary Medicinal Products (CHMP). Note for Guidance on Harmonisation of Requirements for Influenza Vaccines*. (CHMP/BWP/214/96), (adopted March 1997).
55. Govaert, T.M., et al., *Immune response to influenza vaccination of elderly people. A randomized double-blind placebo-controlled trial*. Vaccine, 1994. 12(13):1185-9.
56. Govaert, T.M., et al., *The efficacy of influenza vaccination in elderly individuals. A randomized double-blind placebo-controlled trial*. JAMA, 1994. 272(21): p. 1661-5.
57. Vu, T., et al., *A meta-analysis of effectiveness of influenza vaccine in persons aged 65 years and over living in the community*. Vaccine, 2002. 20(13-14):1831-6.
58. Gross, P.A., et al., *The efficacy of influenza vaccine in elderly persons. A meta-analysis and review of the literature*. Ann Intern Med, 1995. 123(7): p. 518-27.
59. Nichol, K.L., *The efficacy, effectiveness and cost-effectiveness of inactivated influenza virus vaccines*. Vaccine, 2003. 21(16): p. 1769-75.
60. Beyer, W.E., et al., *Protection against influenza after annually repeated vaccination: a meta-analysis of serologic and field studies*. Arch Intern Med, 1999. 159(2): p. 182-8.
61. Barker, W.H., et al., *Communitywide laboratory-based influenza surveillance focused on older persons, 1989-1992*. Am J Prev Med, 1995. 11(3): p. 149-55.
62. Carrat, F. and A.J. Valleron, *Influenza mortality among the elderly in France, 1980-90: how many deaths may have been avoided through vaccination?* J Epidemiol Community Health, 1995. 49(4): p. 419-25.
63. Morens, D.M. and V.M. Rash, *Lessons from a nursing home outbreak of influenza A*. Infect Control Hosp Epidemiol, 1995. 16(5): p. 275-80.
64. Arden, N., A.S. Monto, and S.E. Ohmit, *Vaccine use and the risk of outbreaks in a sample of nursing homes during an influenza epidemic*. Am J Public Health, 1995. 85(3): p. 399-401.

2

Influenza vaccination in community dwelling elderly. Impact on mortality and influenza-associated morbidity

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Abstract

Background

Influenza-related morbidity and mortality have been extensively studied with hospital and reimbursement data. However, little is known about the effectiveness of the annual vaccination programs in generally healthy community-dwelling elderly. The objective of our study was to investigate the effectiveness of influenza vaccination in community-dwelling elderly during the 1996 to 1997 influenza epidemic.

Methods

We performed a population-based cohort study using the computerized Integrated Primary Care Information database in the Netherlands. Subjects who were 65 years and older in 1996 with a permanent status in a practice in the source population were considered eligible for study participation. Two cohorts were defined on the basis of vaccination status. We estimated and compared all-cause mortality, pneumonia, and clinical influenza infection rates between the cohorts.

Results

Influenza vaccination was associated with a significant reduction of morbidity and mortality in vaccinated elderly (relative risk [RR], 0.72; 95% confidence interval [$CI_{95\%}$]: 0.60-0.87). Influenza infections decreased significantly in the vaccinated population (RR, 0.48; $CI_{95\%}$: 0.26-0.91). Mortality was reduced significantly in elderly with comorbidity (RR, 0.67; $CI_{95\%}$: 0.48-0.94). The risk reduction for pneumonia was nonsignificant (RR, 0.77; $CI_{95\%}$: 0.55-1.07) but was temporally related to the peak influenza activity.

Conclusions

In this study, influenza vaccination was associated with decreased mortality and influenza infections in community-dwelling elderly. Our results indicate that, in a season of mild influenza activity and good antigenic match between vaccine strains and circulating strains, influenza vaccination reduced mortality in the vaccinated population. Our data support an annual vaccination strategy for all community-dwelling elderly.

Introduction

Epidemics caused by influenza virus are associated with considerable morbidity and mortality, especially in patients with high-risk conditions [1, 2]. It has been estimated that persons who are 65 years and older account for more than 80% of all pneumonia- and influenza-related deaths [3]. In the Netherlands, the excess mortality caused by influenza is estimated at more than 2000 persons per year. Generally, the increased incidence in influenza-associated mortality is thought to be age related [2].

Although seasonal epidemiologic surveillance of influenza infections is standard practice, effectiveness of influenza vaccination programs is not evaluated systematically. In the published literature, morbidity and mortality data are mostly obtained from hospital records or reimbursement data or are studied in institutionalized elderly [4]. It is likely that institutionalized elderly are less healthy and have a higher risk of mortality and influenza-associated morbidity than community-dwelling elderly. Although in healthy adults influenza vaccination has been shown to be effective, this is not unequivocally shown in patients with asthma or chronic obstructive pulmonary disease [5-7].

In general, little is known about the effectiveness of annual influenza vaccination programs in community-dwelling elderly. One study addressed the issue during one season in a placebo-controlled trial in one general practice [8] and another recent study focused on reduction of mortality of patients admitted to the hospital, but did not include outpatient morbidity and mortality [9]. The need for more data has been expressed before [10].

For regulatory purposes, immunogenicity and safety of the yearly produced inactivated influenza vaccines are studied in small-scale, open, uncontrolled trials [11]. These trials cannot be considered representative of vaccine effectiveness assessment in that particular season or the target population defined by the influenza vaccination programs. For clinical protection in defined target populations, the antigenic match between vaccine strain(s) and circulating strain(s), vaccination coverage, annual revaccination, demographic characteristics, health status, and possible attack rate of the population of interest are at least as important as immune responsiveness.

The influenza season of 1996 to 1997 was of mild severity, with a good match between vaccine strain and the predominant circulating strain (A/H3N2/Nanchang/933/95). A peak activity of influenza-like illness of 28.8 per 10,000 persons was observed in week 4 of 1997, with a distribution in elderly similar to that of the general population [12].

The objective of the present study was to investigate the effectiveness of influenza vaccination in community-dwelling elderly who were 65 years or older during this influenza season.

Methods

Setting

Since 1992, the Integrated Primary Care Information (IPCI) Project at the Department of Medical Informatics of the Erasmus Medical Centre in Rotterdam, the Netherlands, monitors a population of approximately 485,000 patients. The IPCI Project gathers all medical data from 125 general practitioner (GP) practices, including demographic information, patient complaints and symptoms, diagnoses, results of laboratory tests, referral notes from consultants, and hospital admissions. The International Classification for Primary Care is used as the coding system for symptoms and diagnoses [13] but these can also be included as free text. In the database, all prescriptions are recorded and include drug name, anatomical therapeutical chemical code, dosage form, dose, prescribed quantity, and indication. Repeat prescriptions are also recorded. As the system is fully automated, no paper records are held concurrently. Patient and practice identifiers are omitted to ensure anonymity. The system complies with European guidelines on the use of data for medical research and has been shown to be valid for pharmacoepidemiologic research [14].

Design and exposure definition

To assess the effectiveness of influenza vaccination, we conducted a retrospective cohort study. Persons who were 65 years or older in 1996 with a permanent status in one of the practices in the IPCI source population, and who had visited the GP at least once during the study period, were considered eligible for study participation. The study period ran from September 1, 1996, until June 1, 1997, including a 3-month enrolment period and a fixed period of 6 months of follow-up. For every individual who had received an influenza vaccination, 1 age- and sex-matched unvaccinated control subject was randomly selected. To facilitate age matching, the complete eligible population was split into 5-year categories (65-69, 70-74, 75-79, 80-84, and >84 years). The index date was defined as the date of vaccine administration and was thus the start of follow-up. Data on influenza vaccination were gathered from the prescription file (anatomical therapeutical chemical code J07BB) and from free-text notifications in individual patient records. Matched controls had the same index date as their vaccinated counterparts.

Outcome and co-variate definition

All-cause mortality was defined as the primary outcome. Pneumonia and influenza infection without pneumonia were defined as secondary outcomes. In the first analysis, all cohort members were followed up until death or the end of the study period. In a second analysis, they were followed up until pneumonia, a notification of influenza, death, or end of the study period occurred. Influenza was identified by International Classification for Primary Care code R80.0 ("proven infection without pneumonia"). Four disease clusters were identified as covariates that might act as potential confounders: respiratory tract disease (pulmonary emphysema, chronic obstructive pulmonary disease, and asthmatic bronchitis); cardiovascular tract disease and related diagnoses (heart failure, angina pectoris, hypertension, and diabetes mellitus); renal function impairment; and cancer (lung carcinoma, breast carcinoma, prostate carcinoma, colon carcinoma, and other types of malignancy). Their baseline prevalences were derived from diagnosis codes and free-text searches of all available medical history preceding the index date. Confounding by indication for these clusters was anticipated because high-risk patients might be more readily vaccinated. Furthermore, confounding by vaccination status was anticipated because individuals who had received an influenza vaccination in the preceding year might have a different health status and might thus have been more readily vaccinated.

Analyses

For univariate comparisons between proportions we used χ^2-statistics with Yates correction. All tests were 2-sided with a rejection of the null hypothesis at $P<.05$. Continuous variables were compared with a t-test or a Mann-Whitney test when non-normally distributed. The incidence rates of events were calculated by dividing the number of each event by its corresponding person-time. Incidence rates were compared between exposed and non-exposed subjects and expressed as incidence rate ratios with 95% confidence intervals. Confidence intervals for the crude and adjusted incidence rate ratios were calculated with a Cox proportional hazards regression model. Adjusted estimates for incidence rate ratios were calculated with inclusion of all potential confounders at baseline, which were univariately associated with the outcome. Subsequently, the cohort was stratified into persons with risk factors at baseline and persons with no risk factor at baseline. In these strata, incidence rate ratios and the number needed to treat to prevent 1 fatal outcome were calculated. Time-to-event analyses were visualized in life tables. To calculate preventive fractions, we used the following formula:

$(IR_{controls} - IR_{vaccine})/IR_{controls}$, in which IR is the incidence rate. Numbers needed to treat were calculated by the following formula:
$1/[(1 - e^{-IRcontrol \times follow\text{-}up\ time}) - (1 - e^{-IRvaccine \times follow\text{-}up\ time})]$.
Vaccine efficacy was calculated by (1 – RR) x 100%, where RR indicates relative risk.

Results

The IPCI database contained data on 46,610 persons who were 65 years or older in 1996, of whom 20,967 were eligible for study entry, which means that they had a permanent status in one of the practices and had visited the GP at least once during the study period. During the enrollment period, 8911 patients had received an influenza vaccination (42.5% of the eligible population). From the remaining 12,056 non-vaccinated elderly, we selected 8,911 age- and sex-matched controls.

Baseline characteristics of these patients are given in Table 1. Because of the matching procedure, the mean age for the vaccinated (vaccine cohort) and non-vaccinated (control cohort) groups was almost identical. The vaccine and control groups, however, differed significantly in baseline prevalence of underlying diseases. Chronic respiratory diseases, including emphysema and chronic obstructive pulmonary disease, were 77% more frequent in the vaccine cohort. Heart failure and angina pectoris were, respectively, 49% and 31% more frequently noted. Malignancies were 24% more prevalent in the vaccine cohort. The prevalences of diabetes mellitus and hypertension in the vaccine cohort were, respectively, 51% and 11% higher. A greater than 2-fold higher prevalence of renal dysfunction was seen, albeit at a low background incidence. Overall, the prevalence of any serious comorbidity was 25% higher in the vaccine than in the control cohort. There was no substantial difference between the vaccine and control cohorts in duration of the comorbidity. In the vaccine cohort, 3 times as many individuals had been vaccinated against influenza in the preceding year.

Table 2 gives the relative risks of the outcomes of interest in the index and control cohorts. In the total population, vaccination was associated with a significantly lower incidence of pooled events (death, pneumonia, or influenza), all-cause mortality, and influenza infections, after adjustment for respiratory tract disease, cardiac disease, hypertension, diabetes mellitus, renal dysfunction, cancer, and vaccination history. Vaccine efficacy for mortality was 24% ($CI_{95\%:}$ 3%-40%) in the total population and 33% ($CI_{95\%}$: 6%-52%) in the subpopulation with comorbidity. This meant that, in the total population, 1 death was prevented in approximately every 400 vaccinated individuals. In the subpopulation with comorbidity, 1 death was prevented in approximately every 170 vaccinated individuals.

Table 1. Baseline characteristics of the study population

	No. (%)				
Variable	**Vaccine cohort (n=8911)**		**Control cohort (n=8911)**		**RR (± $CI_{95\%}$)**
Sex					
Male	3589	(40.3)	3589	(40.3%)	Reference
Female	5322	(59.7)	5322	(59.7)	1.0 (0.94-1.06)
Age,y					
65-69	2428	(27.2)	2428	(27.2)	
70-74	2372	(26.6)	2372	(26.6)	
75-79	1954	(21.9)	1955	(21.9)	
80-84	1227	(13.8)	1226	(13.8)	
85-89	651	(7.3)	651	(7.3)	
≥ 90	279	(3.1)	279	(3.1)	
Vaccination year before[#]					
No	5851	(65.7)	7538	(84.6)	Reference
Yes	2857	(32.1)	906	(10.2)	*3.06 (2.86-3.27)*
Respiratory system	784	(8.8)	444	(5.0)	*1.77 (1.58-1.98)*
Emphysema/COPD	568	(6.4)	342	(3.8)	*1.66 (1.46-1.89)*
Asthmatic bronchitis	211	(2.4)	175	(2.0)	1.21 (0.99-1.47)
Cardiovascular system/DM	1484	(16.7)	1039	(11.7)	*1.43 (1.33-1.54)*
Heart failure	471	(5.3)	316	(3.5)	*1.49 (1.30-1.71)*
Angina pectoris	309	(3.5)	236	(2.6)	*1.31 (1.11-1.55)*
Cancer	287	(3.2)	232	(2.6)	*1.24 (1.04-1.47)*
Lung carcinoma	41	(0.5)	29	(0.3)	1.41 (0.88-2.27)
Breast carcinoma	104	(1.2)	102	(1.1)	1.02 (0.78-1.34)
Prostate carcinoma	75	(0.8)	55	(0.6)	1.36 (0.96-1.93)
Colon carcinoma	70	(0.8)	50	(0.6)	1.40 (0.98-2.01)
Miscellaneous					
Diabetes mellitus	866	(9.7)	575	(6.5)	*1.51 (1.36-1.67)*
Hypertension	1934	(21.7)	1735	(9.5)	*1.11 (1.05-1.18)*
Renal dysfunction	38	(0.4)	18	(0.2)	*2.11 (1.21-3.70)*
No co-morbidity [#]	5349	(60.0)	6050	(67.9)	Reference
Any co-morbidity [#]	3562	(40.0)	2861	(32.1)	*1.25 (1.20-1.30)*
< 1-y history	904	(10.1)	702	(7.9)	Reference
1- < 2-y history	873	(9.8)	771	(8.7)	0.94 (0.88-1.00)
2- < 3-y history	591	(6.6)	401	(4.5)	1.09 (0.98-1.20)
3- < 4-y history	300	(3.4)	229	(2.6)	1.01 (0.87-1.18)
≥ 4-y history	894	(10.0)	758	(8.5)	0.96 (0.89-1.02)

(#) Less than 1 year history in 1995: 670 persons (3.8%); Co-morbidity: any of the above mentioned types of respiratory-, cardiovascular-, malignant- or miscellaneous diseases; history pertains to the earliest notification of one or more of these diseases in the automated patient profile

Table 2. Crude and adjusted relative risks of death, pneumonia or influenza after influenza vaccination

Outcome	Vaccine cohort		Control cohort		Preventive fraction[(a)]	Crude RR ($CI_{95\%}$)	Adjusted RR ($CI_{95\%}$)
	No.	rate*	No.	rate*			
Total population							
Any event[#]	226	5.3	275	6.5	0.226	*0.83 (0.69-0.99)*	*0.72 (0.60-0.87)*
Death	143	3.3	164	3.8	0.132	0.88 (0.70-1.10)	*0.76 (0.60-0.97)*
Pneumonia	72	1.6	83	1.9	0.158	0.87 (0.63-1.19)	0.77 (0.55-1.07)
Influenza	16	0.4	32	0.7	0.429	*0.51 (0.28-0.94)*	*0.48 (0.26-0.91)*
With co-morbidity							
Any event[#]	122	7.2	115	8.3	0.133	0.86 (0.66-1.11)	*0.73 (0.56-0.96)*
Death	75	4.3	76	5.5	0.214	0.79 (0.57-1.09)	*0.67 (0.48-0.94)*
Pneumonia	44	2.5	29	2.1	-0.190	1.22 (0.76-1.94)	1.08 (0.66-1.76)
Influenza	5	0.3	10	0.7	0.571	0.44 (0.15-1.33)	0.37 (0.12-1.17)
Without co-morbidity (**)							
Any event[#]	104	4.1	160	5.6	0.268	*0.74 (0.58-0.95)*	*0.71 (0.55-0.92)*
Death	68	2.6	88	3.0	0.133	0.89 (0.65-1.23)	0.87 (0.62-1.20)
Pneumonia	28	1.1	54	1.8	0.389	*0.58 (0.37-0.92)*	*0.56 (0.35-0.89)*
Influenza	11	0.4	22	0.7	0.429	0.56 (0.27-1.16)	0.55 (0.26-1.17)

(#) Any event was defined as either death or pneumonia or influenza whichever came first

(*) Incidence rate expressed as number of cases per 100 patient-years

[(a)] Preventive fraction calculated as $(IR_{controls} - IR_{index\ cases})/IR_{controls}$

($) In the total population and the subpopulation with co-morbidity: adjusted for respiratory tract disease, cardiac disease, hypertension, diabetes mellitus, renal dysfunction and vaccination history

(**) In the population without co-morbidity: only adjusted for vaccination history

Figure 1. Time to event analysis - Pneumonia

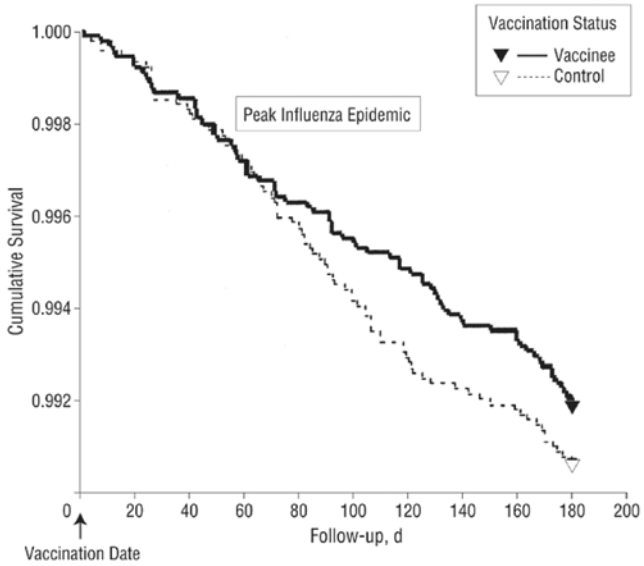

Table 3. Adjusted relative risks of death, pneumonia or influenza after influenza vaccination, stratified by age and gender

Outcome	Adjusted RR* (+/-$CI_{95\%}$)
Males	
Any event#	*0.67 (0.52-0.87)*
Death	*0.71 (0.51-0.99)*
Females	
Any event#	0.79 (0.60-1.03)
Death	0.83 (0.59-1.16)
65-74 years of age	
Any event#	*0.67 (0.49-0.93)*
Death	0.70 (0.43-1.14)
75-84 years of age	
Any event#	0.78 (0.58-1.04)
Death	0.78 (0.54-1.11)
≥85 years of age	
Any event#	0.76 (0.52-1.09)
Death	0.88 (0.58-1.33)

(#) Any event was defined as either death or pneumonia or influenza whichever came first

(*) Adjusted for respiratory tract disease, cardiac disease, hypertension, diabetes mellitus, renal dysfunction and vaccination history

In the subpopulation with comorbidity, vaccination was associated with a significant risk reduction of death but not pneumonia. The reduced risk of influenza infection in this subpopulation was nonsignificant. In the absence of comorbidity, relative risks adjusted for revaccination status showed a risk reduction for the pooled events and for pneumonia. For the total population, the risk reduction for pneumonia was visualized in a life table as the time to event (Figure 1). Risk reduction became prominent approximately 2 months after vaccination (i.e., the first weeks of 1997, the time of peak influenza activity).

Finally, effect modification by age and sex is shown in Table 3. Only in the age category 65 to 74 years was vaccination associated with a significant reduction of all events, and effectiveness decreased with increasing age; in addition, men seemed to benefit more than women from influenza vaccination.

Comment

In our study, influenza vaccination effectively prevented mortality and morbidity in elderly under everyday circumstances in a season with a good match between vaccine strain(s) and circulating strain(s). Although in individuals with comorbidity, influenza vaccination failed to show a protective effect against pneumonia, mortality was significantly reduced. It is known that most of the excess mortality caused by

influenza and/or pneumonia is attributed to elderly with high-risk conditions [15]. Possibly, elderly with an impaired clinical condition die as a result of influenza before developing pneumonia, or pneumonia is not recognized because symptoms in the elderly may be less prominent.

Our data suggest that the effectiveness of influenza vaccination declines with age. This has also been shown in a randomized, placebo-controlled trial in healthy elderly followed up in one GP practice [8]. As in our study, that trial showed a risk reduction of approximately 50% in clinical influenza in healthy elderly. Observational cohort studies mostly address influenza-related hospital admissions or focus on elderly in institutions or with high-risk conditions [4, 16-18]. In a recent large-scale cohort study [9] in community-dwelling elderly, a significant risk reduction of hospital admission–associated mortality was observed. Limitations of that study, however, were that outpatient mortality and morbidity were not included and that potentially confounding cofactors were not adjusted for.

Annual influenza vaccination has been proposed as a strategy to increase effectiveness [19-20]. In institutionalized elderly, annual revaccination as well as vaccination coverage was shown to be the most consistent contributors to survival [21-22]. This is supported by evidence from a meta-analysis [23]; however, the evidence has been questioned [24]. Also in our study, preliminary assessment of annual revaccination appeared to enhance survival, although numbers were too small in the different subpopulations to allow for appropriate conclusions.

Epidemiologic studies under everyday circumstances may suffer from selection bias, information bias, and confounding. As GPs play a central role in the Dutch health care system and cover the complete population, selection bias was highly unlikely. Also, information bias was unlikely, since influenza vaccination was recorded by computer, the vaccination is supplied almost exclusively by GPs in the Netherlands, and the primary outcome "mortality" is difficult to misclassify. Misclassification of pneumonia is probably modest; all cases were confirmed radiologically and/or microbiologically. However, it is possible that influenza was underestimated in the vaccine cohort because physicians were aware of the vaccination status of their patients. A potential problem was confounding by indication. Although the national recommendation was to vaccinate those who were 65 years and older, generally healthy individuals do not always follow such advice. In our study, confounding by indication was likely, since pre-existing respiratory or cardiovascular tract disease were independent risk factors for increased mortality, as was a history of influenza vaccination. These factors were thus adjusted for. As we may not be aware of all comorbidity, residual confounding cannot be excluded. However, this probably means that our estimates are conservative and that true protection may be higher.

In conclusion, our results indicate that, in a season of mild influenza activity and good antigenic match between vaccine strain(s) and circulating strain(s), influenza vaccination reduces influenza-related morbidity and all-cause mortality in community-dwelling elderly. The decrease in mortality was most prominent in elderly with high-risk conditions. Vaccine efficacy, calculated as a risk reduction in mortality of 33% in this subpopulation, could be translated to approximately 170 vaccinated individuals to prevent 1 death. Hence, our data suggest that a national policy to vaccinate all those who are 65 years and older against influenza can be successful. We argue that the assessment of the benefits of annual influenza vaccination programs should focus on survival and primary and secondary influenza-related morbidity in community-dwelling elderly. Such data may improve the cost-effectiveness estimates of influenza vaccination programs, where health care resource use includes GP visits and drug use in addition to hospitalizations [25].

References

1. Murray CJL, Lopez AD. *Mortality by cause for eight regions of the world: Global Burden of Disease Study.* Lancet 1997;349:1269-76
2. Sprenger MJW, Mulder PG, Beyer WE, van Strik R, Masurel N. *Impact of influenza on mortality in relation to age and underlying disease, 1967-1989.* Int.J.Epidemiol.1993,22:334-40
3. From the CDC (MMRW 1995;44:535-7). *Pneumonia and influenza death rates - United Stated 1979-1994.* JAMA,1995,247;7:532
4. Gross, PA, Hermogenes, AW, Sacks HS, Lau J, Levandowski RA. *The efficacy of influenza vaccine in elderly persons. A meta-analysis and review of the literature.* Ann Intern Med, 1995. 123(7): p. 518-27.
5. Buxton Bridges C, Thompson WW, Meltzer MI, et al. *Effectiveness and cost-benefit of influenza vaccination of healthy working adults. A randomized controlled trial.* JAMA, 2000, 284; 13:1655-63
6. Nichol KL, Lind A, Margolis KL, et al. *The effectiveness of vaccination against influenza in healthy working adults.* N.Engl.J.Med.1995, 333;14:889-93
7. Hak E. *Effectiveness and implementation of influenza vaccination. A non-experimental approach. Chapter 7: Lack of effectiveness of conventional influenza vaccination among patients with asthma of COPD of working age.* Thesis, September 11, 2001
8. Govaert ThME, Thijs MCTMCN, Masurel N, Sprenger MJW, Dinant GJ, Knottnerus JA. *The efficacy of influenza vaccination in elderly individuals. A randomized double blind placebo controlled trial.* JAMA, 1994,272; 21: 1661-5
9. Christenson B, Lundbergh P, Hedlund J, Ortqvist A. *Effects of a large-scale intervention with influenza and 23-valent pneumococcal vaccines in adults aged 65 years or older: a prospective study.* Lancet, 2001; 357: 1008-11
10. Carrat F, Tachet A, Housset B, Valleron AJ, Rouzioux C. *Influenza and influenza-like illness in general practice: drawing lessons for surveillance from a pilot study in Paris, France.* Br.J.Gen.Pract. 1997, 47; 417: 217-20.
11. CHMP/BWP/214/96. *Note for guidance on harmonization of requirements for influenza vaccines.* 12 march 1997
12. Rimmelzwaan GF, de Jong JC, Bartelds AI, Claas EC, van Wijngaarden JK, Osterhaus AD. *Influenza in het seizoen 1996/'97: vaccine samenstelling voor het seizoen 1997/'98.* NTvG 1997,141;36:1743-7
13. Lamberts H, Woods M. *International Classification of Primary Care.* Oxford, UK: Oxford University Press 1987
14. Vlug AE, van der Lei J, Mosseveld BMTh, et al. *Postmarketing surveillance based on electronic patient records: The IPCI project.* Meth.Inform.Med. 1999, 38:339-44
15. Barker WH, Mulloolly JP. *Pneumonia and influenza deaths during epidemics. Implications for prevention.* Arch.Int.med. 1982,142:85-9
16. Nichol KL, Baken L, Nelson A. *Relation between influenza vaccination and outpatient visits, hospitalisation and mortality in elderly persons with chronic lung disease.* Ann. Int. Med. 1999, 130:397-403
17. Nichol KL, Margolis KL, Wuorenma, J, Von Sternerg T. *The efficacy and cost-effectiveness of vaccination against influenza among elderly persons living in the community.* N.Engl.J.Med. 1994, 31: 778-84
18. Barker WH, Mullooly JP. *Influenza vaccination of elderly persons. Reduction in pneumonia and influenza hospitalisations and deaths.* JAMA 1980,244:2547-9
19. McElhaney JE, Meneilly GS, Beattie BL, et al. *The effect of influenza vaccination on IL2 production in healthy elderly: implications for current vaccination practice.* J.Gerontol.1992, 47; 1: M3-8

20. Ahmed AH, Nicholson KG, Nguyen-van Tam JS. *Reduction in mortality associated with influenza during the 1989-1990 epidemic*. Lancet, 1995,346:591-5
21. Saah AJ, Neufeld R, Rodstein M, et al. *Influenza vaccine and pneumonia mortality in a nursing home population*. Arch.Int.Med. 1986,146:2353-7
22. Arden N, Monto AS, Ohit SE. *Vaccine use and the risk of outbreaks in a sample of nursing homes during an influenza epidemic*. Am.J.Publ.Health 1995,85; 3: 399-401
23. Beyer, WE, de Bruijn IA, Palache AM, Westendorp RG, Osterhaus AD. *Protection against influenza after annually repeated vaccination: a meta-analysis of serologic and field studies*. Arch Intern Med, 1999, 159;2:182-8.
24. Hoskins, TW, Davies JR, Smith AJ, Miller CL, Allchin A. *Assessment of inactivated influenza-A vaccine after three outbreaks of influenza A at Christ's Hospital*. Lancet, 1979, 1; 8106: 33-5.
25. Postma JM, Bos JM, van Gennep, M, Jager JC, Baltussen R, Sprenger MJW. *Economic evaluation of influenza vaccination*. Pharmacoeconomics 1999,16; suppl.1: 33-40

3

Annual revaccination against influenza and mortality risk in community dwelling elderly persons

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Abstract

Context

Although large-scale observational studies have demonstrated the effectiveness of influenza vaccination, no large studies have systematically addressed the clinical benefit of annual revaccinations.

Objective

To investigate the effect of annual influenza revaccination on mortality in community-dwelling elderly persons.

Design, Setting, and Participants

A population-based cohort study using the computerized Integrated Primary Care Information (IPCI) database in the Netherlands including community-dwelling individuals aged 65 years or older from 1996 through 2002. For each year, we computed the individual cumulative exposure to influenza vaccination since study start.

Main Outcome Measure

Association between the number of consecutive influenza vaccinations and all-cause mortality vs. no vaccination after adjusting for age, sex, chronic respiratory and cardiovascular disease, hypertension, diabetes mellitus, renal failure, and cancer.

Results

The study population included 26,071 individuals, of whom 3,485 died during follow-up. Overall, a first vaccination was associated with a nonsignificant annual reduction of mortality risk of 10% (hazard ratio [HR], 0.90; 95% confidence interval [$CI_{95\%}$], 0.78-1.03) while revaccination was associated with a reduced mortality risk of 24% (HR, 0.76; $CI_{95\%}$: 0.70-0.83). Compared with a first vaccination, revaccination was associated with a reduced annual mortality risk of 15% (HR, 0.85; $CI_{95\%}$: 0.75-0.96). During the epidemic periods this reduction was 28% (HR, 0.72; $CI_{95\%}$: 0.53-0.96). Similar estimates were obtained for persons with and without chronic comorbidity and those aged 70 years or older at baseline. Overall, influenza vaccination is estimated to prevent 1 death for every 302 vaccinees at a vaccination coverage that varied between 64% and 74%.

Conclusion

Annual influenza vaccination is associated with a reduction in all-cause mortality risk in a population of community-dwelling elderly persons, particularly in older individuals

Introduction

Influenza-associated morbidity and mortality increase with age, especially for individuals with high-risk conditions [1,2]. The estimated impact of annual influenza epidemics on morbidity and mortality on elderly persons and the effectiveness of influenza vaccination have been the basis for implementing nationwide influenza vaccination programs for elderly individuals [3].

The effectiveness of vaccination has been reported to decrease in high-risk persons [4-7]. Annual influenza revaccination has been proposed as a strategy to increase vaccination effectiveness [8-11]. However, clinical studies have not always shown a consistent benefit of annual revaccination. In institutionalized elderly persons, annual revaccination resulted in improved survival [12-13], whereas, in a placebo-controlled trial among 1,838 community-dwelling elderly persons, prior vaccination did not further reduce the occurrence of clinical influenza [4]. In a trial of healthy adults (aged 30-60 years), annual influenza vaccination had no additional effect on the risk of clinically diagnosed influenza like illness, although both first vaccination and repeat vaccination showed a greater decrease in virus shedding and better annual protection against influenza virus infection compared with placebo [14-15]. In a clinical trial among boarding school students, revaccination did not confer any benefit with respect to serologically or virologically confirmed influenza [16]. A recent meta-analysis comparing single and multiple vaccinations showed that although 7 of 10 field trials supported sustained protection against laboratory-confirmed influenza like illness upon revaccination, the pooled rate difference of 1.1% was not significant [17].

Recommendations regarding annual vaccination are often based on the reported influenza-attributed mortality and morbidity and effectiveness of vaccination without systematic data on revaccination status [18-21]. So far, studies to establish the effectiveness of repeated influenza vaccinations within the scope of national programs have not been performed in a population-based setting.

Our objective was to investigate the relationship of influenza revaccination status on mortality in community-dwelling persons aged 65 years or older during the epidemic influenza seasons covering 1996-2002

Methods

Setting

In the Netherlands, a nationwide influenza vaccination program has been active since 1997. In the Dutch health care system, all persons are designated to their own general practitioner (GP) who files all relevant medical details on patients from primary care visits, hospital admissions, laboratory examinations, and visits to outpatient clinics. The vaccination program is executed by GPs during annual mass vaccination days in October and November, during which all individuals aged 65 years and older and adults and children with predefined risk factors are invited to participate in the vaccination campaign. General Practitioners register the vaccination date in the electronic patient record.

Since 1994, the Integrated Primary Care Information (IPCI) Project at the Department of Medical Informatics of the Erasmus Medical Center in Rotterdam, the Netherlands, has assembled electronic patient records on a cumulative population of approximately 500,000 patients from approximately 150 GPs. The IPCI database is a general practice research database that contains information on all medical data, including demographic information, patient complaints and symptoms, diagnoses, results of laboratory tests, referral notes from consultants, and hospital admissions.

The International Classification for Primary Care is used as the coding system for symptoms and diagnoses [22] but these can also be included as free text. All prescriptions are recorded in the database, which includes drug name, Anatomical Therapeutical Chemical (ATC) code, dosage form, dose, prescribed quantity, and indication. The IPCI database is the sole repository of medical records, and no additional paper records of the patients are kept by the GPs. Patients and practice identifiers are altered to warrant anonymity. The system complies with European Commission guidelines on the use of data for medical research and has shown to be valid for pharmacoepidemiologic research [6, 23]. The IPCI internal review board approved the project and patient consent was not required.

Study population

In the IPCI database 49,818 individuals were 65 years or older at any time during the study period. First, we excluded all practices that did not consistently register influenza vaccination over the study years. Non-consistent registration was defined as a difference between minimum and maximum annual vaccination coverage of at least 50% and / or a minimum vaccination coverage recording of less than 25%.

After exclusion, 34,991 persons remained. In this remaining study population, we conducted a cohort study during the period between October 1, 1996, and September 30, 2002.

We included patients who were 65 years or older on January 1 of the year of study start, who had a permanent status in 1 of the practices in the IPCI source population, and who had at least 1 year of recorded database history prior to study start to determine heath status and vaccination status. We excluded 8,920 individuals who did not have a recorded database history in the GP practice of 1 year or more. The eligible population thus included 26,071 persons. Censoring was performed at death, moving out of the GP practice, or at the end of the study period, whichever came first.

Exposure definition

The cumulative number of influenza vaccinations was determined between October 1 and December 31 of each calendar and was assigned to each individual on January 1 of the next year. This date was chosen to compensate for the slight variability in vaccination dates, mostly between late October and early December, and the lag time before vaccination becomes effective. (An additional analysis using actual date of vaccination did not substantively affect the results.)

Exposure status was categorized into 9 mutually exclusive categories including non-exposed, first vaccination, second, third, fourth, fifth, and sixth (or seventh) vaccination, vaccination interruption, and restart. A first vaccination status was assigned to individuals who received the first vaccination after study entry with no recorded influenza vaccination prior to study entry. If persons were vaccinated prior to study entry they started with the number of previously recorded vaccinations. Upon each additional consecutive vaccination during the study period, the cumulative number of influenza vaccinations increased by one. When a vaccination series was interrupted, it was categorized as such. Finally, restart after 1 or more years of interruption was also categorized separately. Once in the interruption category, individuals remained in it until vaccination restart and vice versa. Consequently, in this time-varying approach of exposure analysis, individuals contributed information to different exposure categories during follow-up.

Outcome definition

The primary outcome in this study was all-cause mortality. Death was identified from the demographic patient file and validated in the medical chart. Deaths occurring during the period January 1 and December 31 were allocated to the vaccination status defined in the period between October 1 and December 31 of the preceding year.

In an extra analysis, we compared mortality during the epidemic periods (defined as the first day of the first week of the recorded epidemic until the last days of the last week of the recorded epidemic) with a reference period during the summer months (July and August).

Co-variates

Selection of covariates was based on an earlier study from our group in the same database [6]. In addition to age, sex, and epidemic year, we identified 6 disease clusters as potential confounders: chronic respiratory tract disease (chronic obstructive pulmonary disease, emphysema, chronic bronchitis, asthma); chronic cardiovascular disease (heart failure, angina pectoris, history of myocardial infarction or cerebrovascular accident, aortic aneurysm, chronic arterial dysfunction); hypertension; diabetes mellitus; chronic renal insufficiency; and malignancies. The presence of these conditions at study entry or their development at any time during follow-up was retrieved from the medical charts through automatic screening and further manual validation. Those who had no comorbidity at baseline and did not develop any of the predefined conditions during follow-up were considered as the population without comorbidity.

Information on the size of each influenza epidemic was obtained from Jan C. de Jong, PhD, of the National Influenza Center, Erasmus MC Rotterdam (written communication, August 21, 2003, and March 5, 2004) [24].

Analyses

To estimate the univariate association between vaccination, covariates, and death we used a Cox proportional hazards model. Multivariate time-varying Cox proportional hazard models were developed to estimate the hazard ratios (HRs) for different vaccination states while adjusting for all other risk factors [25]. In most analyses the non-exposed category was used as reference category. For the estimation of the HRs of revaccination vs. first vaccination, the first vaccination was used as reference category. To fully adjust for the strong influence of age on death in this analysis, we used age in days as time axis. The exposure status of an individual on the date of death was compared with all individuals in the cohort on that moment during follow-up on which they had exactly the same age as the individual who died. We also adjusted for sex and for time since the beginning of the study to adjust for epidemic year. To adjust for comorbidity that occurred during follow-up, time-dependent covariates were used for the diseases defined above.

The association of vaccine exposure with mortality risk was evaluated in 3 analyses: any vaccination vs. no previous vaccination; a first vaccination, revaccination, interruption, and restart vs. no vaccination; and any revaccination vs. a first vaccination. Subsequently, revaccination was further analyzed by second, third, fourth, fifth, and sixth or seventh vaccination.

Stratified analyses were conducted on the presence of comorbidity and age at study entry (65-69, 70-79, or >79 years).

All results were expressed as HRs with 95% confidence intervals ($CI_{95\%}$). In the total population, numbers needed to vaccinate to save 1 death were calculated as: $1/[(1-e^{-IRcontrol \times follow\text{-}up\ time}]-[1-e^{-IRindex \times follow\text{-}up\ time}])$, where IR is the incidence rate.

All analyses were performed using SAS software, version 8.2 using the procedure Proc Phreg. Statistical significance was set at $P<0.05$.

Results

Baseline characteristics of the population and univariate associations of covariates with all-cause mortality are provided in Table 1. Of the 26,071 persons who were eligible for study entry, 3,485 died during follow-up. The mean duration of participation in the study was 3 years. The mean (SD) age at study entry was 73.1 (7.4) years and 58% were women. At baseline 53.3% of the population had some form of comorbidity, mostly hypertension (24.6%) and chronic cardiovascular diseases (23.4%). Mortality was strongly associated with age, sex, and comorbidity. The mortality rate was highest for individuals with chronic renal dysfunction or malignancies.

The vaccination coverage and vaccination status for each study year are shown in Table 2. During the total study period, the population studied received 62,476 influenza vaccinations. Ninety-six percent of the vaccinations were given in October or November, and 3.6% in December. The annual vaccination coverage ranged from 64% in 1996 to 74% in 1999. A total of 5,095 eligible individuals (19.5%) never received influenza vaccination during follow-up.

Influenza epidemics during the study period were of mild to moderate severity (Table 3); the 2000-2001 season showed no clear epidemic activity. Generally, vaccine strains and the predominant circulating strain (mainly A/H3N2) were well matched except for the 1997-1998 season [24]. The peak activity of influenza like illness ranged between 7 cases per 10,000 persons (2000-2001 season) and 32 cases per 10,000 persons per week (1999-2000 season) and was observed between weeks 2 and 13.

Table 1: Characteristics of the study population at study entry

Variable	No. (%)				Risk of dying during follow-up
	Population characteristics at study entry		Deaths in Follow-up		
	(n=26071)		(n=3485)		Univariate HR ($CI_{95\%}$)
Sex					
Female	15131	(58.0)	1818	(12.0)	Reference
Male	10940	(42.0)	1667	(15.2)	1.27 (1.19-1.35)
Age, y					
65-69	10490	(40.2)	537	(5.1)	Reference
70-74	5863	(22.5)	608	(10.7)	
75-79	4669	(17.9)	747	(16.0)	
80-84	2761	(10.6)	686	(24.8)	1.77 (1.73-1.81)
≥ 85	2288	(8.8)	907	(39.6)	
Year of study entry					
1996	10195	(39.1)	2089	(20.5)	Reference
1997	1325	(5.1)	142	(10.7)	
1998	4667	(17.9)	493	(10.6)	
1999	7178	(27.5)	664	(9.3)	0.66 (0.65-0.68)
2000	1528	(5.9)	70	(4.6)	
2001	1178	(4.5)	27	(2.3)	
Co-morbidity					
None at study entry	12173	(46.7)	1034	(8.5)	Reference
Co-morbidity at study entry#	13898	(53.3)	2451	(17.6)	2.15 (2.00-2.31)
Hypertension	6414	(24.6)	837	(13.0)	0.94 (0.87-1.01)
Diabetes mellitus	3000	(11.5)	583	(19.4)	1.59 (1.46-1.74)
Respiratory system	3487	(13.3)	689	(19.8)	1.69 (1.57-1.84)
Cardiovascular system	6099	(23.4)	1378	(22.6)	2.30 (2.15-2.46)
Cancer	1034	(4.0)	330	(31.9)	2.81 (2.50-3.15)
Renal dysfunction	221	(0.8)	87	(39.4)	3.41 (2.75-4.21)

Abbreviations: $CI_{95\%}$: 95% confidence interval; HR: Hazard ratio

(#) During the follow-up period comorbidity developed in another 2903 individuals resulting in a total of 16701 subjects with comorbidity at baseline or any time during follow-up.

In the total population, any vaccination was associated with a 22% lower risk of all-cause mortality (adjusted HR, 0.78; $CI_{95\%}$: 0.72-0.85; Table 4). First vaccination was associated with a nonsignificant reduction in mortality risk of 10% in the total population (adjusted HR, 0.90; $CI_{95\%}$: 0.78-1.03). Any revaccination was associated with a risk reduction of approximately 24% (adjusted HR, 0.76; $CI_{95\%}$: 0.70-0.83), which was strongest during the epidemic period (adjusted HR, 0.72; $CI_{95\%}$: 0.59-0.89) and was not significant during a reference summer period (July and August; adjusted HR, 0.89; $CI_{95\%}$:0.70-1.12).

Table 2: Vaccination coverage and vaccination status per influenza epidemic season

	1996-1997	1997-1998	1998-1999	1999-2000	2000-2001	2001-2002#
Eligible population*						
Total	10195	10991	14302	19676	17234	16590
With comorbidity	5445	6382	8532	11721	10571	10426
Total died	487	490	647	821	711	329
Vaccination coverage, No. (%)*/**						
Not vaccinated	3655 (35.9)	3676 (33.4)	4608 (32.5)	5130 (26.1)	4567 (26.5)	4831 (29.1)
Vaccinated	6540 (64.1)	7315 (66.6)	9694 (67.5)	14546 (73.9)	12667 (73.5)	11759 (70.9)
With comorbidity	3906 (71.7)	4664 (73.1)	6245 (73.2)	9249 (78.9)	8218 (77.9)	7894 (75.7)
Vaccination status						
No vaccination	3655	2707	3130	4020	3114	2955
1st	3030	1497	1061	849	824	395
2nd ##	3510	2661	3181	5477	1106	969
3rd	-	3157	2390	2783	4361	996
4th	-	-	2832	2175	1667	3937
5th	-	-	-	2595	1588	930
6th or 7th	-	-	-	-	2123	3284
Interrupted	-	969	1523	1110	1453	1876
Restarted	-	-	185	667	998	1248

Abbreviations: Elllipses indicate no data

(#) Follow up in the 2001-2002 season ended in September.

(*) With comorbidity at baseline; a proportion of subjects developed comorbidity during follow-up (see table 3)

(**) Percentage in parentheses indicates the proportion of subjects with comorbidity being vaccinated

(##) May also include multiple vaccinations of subjects who only had 1-year history available before study entry and who were vaccinated in that year. For these subjects, it is not known if they had any previous vaccinations.

Compared with a first vaccination, revaccination was associated with a significantly reduced mortality risk of 15% (adjusted HR, 0.85; $CI_{95\%}$: 0.75-0.96). During the epidemic period this risk reduction was 28% (HR, 0.72; $CI_{95\%}$: 0.53-0.96).

When each individual vaccination was modelled separately, the mortality risk showed a decreasing trend with additional consecutive vaccinations (Figure). Interruption of the vaccination series was associated with a strong and significant increase in mortality risk (adjusted HR, 1.25; $CI_{95\%}$: 1.10-1.42). When the vaccination series was interrupted for more than 1 year, this risk estimate increased further, although it was no longer significant (adjusted HR, 1.83; $CI_{95\%}$: 0.94-3.78). Restarting vaccination after an interruption resulted in a mortality risk reduction similar to that observed following revaccination.

Table 3: Epidemiological characteristics of the influenza epidemic seasons*

	Vaccine strains			Predominant epidemic strain(s)	Antigenic match#	Epidemic period (weeks)			
	A/H3N2	A/H1N1	B			Start	Peak	End	Peak ILI$
1996-1997	Wuhan/353/95	Singapore/6/86	Beying/184/93	A/H3N2	+++	2	4	8	29
1997-1998	Wuhan/353/95	Bayern/7/95	Beying/184/93	A/H3N2	+	8	13	15	18
1998-1999	Sydney/5/97	Beying/262/95	Beying/184/93	A/H3N2	+++	6	8	11	22
1999-2000	Sydney/5/97	Beying/262/95	Beying/184/93	A/H3N2	+++	51	2	5	32
2000-2001	Moscow/10/99	N.Caladonia/20/99	Beying/184/93	B	+++	1	4	8	7
2001-2002	Moscow/10/99	N.Caladonia/20/99	Sichuan/379/99	A/H3N2	+++	2	9	12	13

Information in this Table was provided by Jan D de Jong, PhD.

N.Caledonia indicates New Caledonia

(#) +: indicates poor match (some cross protection); ++: fair match (moderate cross protection); +++: good match (substantial cross protection); ++++: excellent match (identical strains or minimal differences) [21]

($) Influenza like illness (ILI) was defined as prodromal phase with feverishness plus at least one of the following symptoms: cough, coryza, soar throat, frontal headache, retrosternal pain, myalgia.

(*) peak ILI denotes the maximum number of cases of ILI per week per 10,000 inhabitants in the Netherlands as reported by the GPs participating in the Continuous Morbidity Registration system of NIVEL (Netherlands Institute for Primary Health Care) during the peak of the epidemic

In the total population 1 death was prevented for every 302 vaccinations, or 1 for every 195 revaccinations.

Exclusion of the population with a history of vaccinations prior to study entry did not change the effect estimates (data available on request). Stratification for comorbidity showed that the largest effects following any vaccination and revaccination were observed in the subpopulation without comorbidity (Table 4). Revaccination was not associated with a reduction in mortality risk in persons aged 65 through 69 years at baseline (adjusted HR, 0.98; $CI_{95\%}$: 0.78-1.23) but was significantly reduced in persons aged 70 through 79 years at baseline (adjusted HR, 0.78; $CI_{95\%}$: 0.68-0.91), and persons aged 80 years and older at baseline (adjusted HR, 0.69; $CI_{95\%}$: 0.61-0.78).

This age-related difference following revaccination seemed to reflect age-related differences in causes of death. In the highest age groups, relatively more individuals died from causes that may be more likely to be influenced by influenza vaccination, such as infectious causes (HR following vaccination, 0.58; $CI_{95\%}$: 0.43-0.79) or old age or "frailty" (HR following vaccination, 0.63; $CI_{95\%}$: 0.55-0.73; Table 5).

We assessed the possibility of confounding by indication, ie. the possibility that those who were not vaccinated were sicker than those who were. However, in our population, the proportion of the population with comorbid illnesses was 50.9% for those with no previous vaccination, 55.8% for those who refused (34% of all those who were not vaccinated), 68.9% for those who had an interruption, and 68.5%

Table 4: Annual adjusted hazard ratios of death, stratified by comorbidity and age

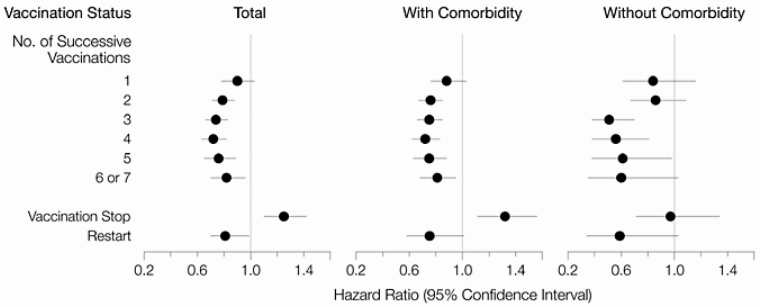
		HR (+/-$CI_{95\%}$)	
	Deaths	**Crude**	**Adjusted***
	No. of cases		
Total population			
Any vaccination	2225	*0.91 (0.84-0.99)*	*0.78 (0.72-0.85)*
First vaccination	284	0.97 (0.85-1.12)	0.90 (0.78-1.03)
Revaccination	1941	*0.90 (0.83-0.98)*	*0.76 (0.70-0.83)*
Vaccination interruption	366	1.43 (1.26-1.62)	1.25 (1.10-1.42)
Vaccination restart	121	0.91 (0.75-1.11)	*0.81 (0.67-0.99)*
Population without co-morbidity#			
First vaccination	47	0.86 (0.62-1.18)	0.84 (0.60-1.16)
Revaccination	217	*0.66 (0.54-0.80)*	*0.66 (0.54-0.80)*
Population with co-morbidity			
First vaccination	237	0.91 (0.78-1.06)	0.88 (0.76-1.03)
Revaccination	1724	*0.82 (0.75-0.90)*	*0.75 (0.68-0.83)*
Age at baseline			
65-69 years			
First vaccination	56	1.20 (0.87-1.66)	1.11 (0.81-1.53)
Revaccination	300	*1.25 (1.00-1.56)*	0.98 (0.78-1.23)
70-79 years			
First vaccination	109	1.02 (0.81-1.28)	0.93 (0.75-1.17)
Revaccination	803	0.95 (0.83-1.10)	*0.78 (0.68-0.91)*
≥ 80 years			
First vaccination	119	0.87 (0.70-1.07)	*0.81 (0.66-1.00)*
Revaccination	838	*0.78 (0.69-0.88)*	*0.69 (0.61-0.78)*

(*) Adjusted for comorbidity (respiratory tract disease, cardiac disease, hypertension, diabetes mellitus, renal dysfunction and malignancy) and gender. Age adjustment by age in days as time axis.

(#) Population without co-morbidity: No recorded predefined present at comorbidity at baseline or developing at any time during follow up.

for those who had any vaccination in series, suggesting that those who were not vaccinated were at least as healthy as those who were. Furthermore, compared with nonusers who refused vaccination, the adjusted mortality risk following the first vaccination was an HR of 1.09 ($CI_{95\%}$: 0.93-1.28) and following revaccination, an HR of 0.93 ($CI_{95\%}$: 0.83-1.04). Compared with those who were not vaccinated and did not refuse, the adjusted HR for mortality following the first vaccination was 0.73 ($CI_{95\%}$: 0.63-0.85) and following any revaccination, 0.62 ($CI_{95\%}$: 0.56-0.70).

Figure. Hazard ratios for mortality by individual vaccination states stratified by population



Mortality risk is shown by the number of successive vaccinations, i.e. first, second, third, fourth, fifth, more then 6, interruption of vaccination (stop) or restart. The hazard ratio indicates the mortality risk following vaccination versus no previous vaccination.

Table 5: Annual adjusted hazard ratios of cause- specific death

Cause of death	No.	age at death mean (SD)	Adjusted Hazard Ratio* ± $CI_{95\%}$ Any vaccination	First vaccination	Revaccination
Overall	3485	81.5 (8.1)	*0.78 (0.72-0.85)*	0.90 (0.78-1.03)	*0.76 (0.70-0.83)*
Cardiovascular	726	82.1 (7.9)	0.89 (0.73-1.08)	0.89 (0.65-1.23)	0.89 (0.73-1.08)
Chronic respiratory disease	180	80.1 (7.5)	1.13 (0.71-1.79)	1.30 (0.67-2.51)	1.11 (0.70-1.77)
Malignancies	549	77.8 (7.4)	0.87 (0.71-1.08)	0.96 (0.68-1.33)	0.85 (0.68-1.05)
Infections	249	84.2 (7.5)	0.58 (0.43-0.79)	*0.56 (0.31-1.00)*	*0.58 (0.43-0.79)*
Diabetes mellitus	33	82.2 (8.9)	1.98 (0.60-6.58)	1.51 (0.25-9.14)	2.02 (0.60-6.76)
Renal insufficiency	34	80.0 (7.2)	1.23 (0.41-3.68)	2.25 (0.55-9.20)	1.11 (0.36-3.38)
"Natural death"	1087	83.8 (8.2)	0.63 (0.55-0.73)	0.83 (0.65-1.05)	*0.61 (0.52-0.70)*
Acute death	458	79.2 (7.2)	0.82 (0.65-1.03)	1.05 (0.73-1.50)	*0.79 (0.62-1.00)*
Unnatural causes	169	79.8 (8.3)	1.11 (0.73-1.67)	1.02 (0.53-1.98)	1.12 (0.73-1.70)

(*) Adjusted for comorbidity (respiratory tract disease, cardiac disease, hypertension, diabetes mellitus, renal dysfunction and malignancy) and gender. Age adjustment by age in days as time axis.

Cardiovascular: Cerebro-vascular accident, heart failure, cardiac asthma
Chronic respiratory: empfysema, ARDS, exacerbation COPD, respiratory failure
Infections: pneumonia, sepsis, septic shock, urosepsis
Natural death: Alzheimer, Parkinson's disease, no specific diagnosis, decubitus, "natural death", old age with other no obvious reason
Acute death: validated sudden cardiac death retrieved from other study in same patient population, found dead without pre-exiting cause
Unnatural causes: euthanasia, "unnatural death", accident, post operative death, preoperative death, dehydration, Creutzfeld Jacobs disease, gastro-intestinal bleeding, murder,

Comment

In this study, we showed that influenza vaccination is associated with a reduced risk of mortality in community dwelling elderly despite several mild epidemic seasons, and that revaccination is an effective strategy to further reduce or sustain reduced mortality risk in both healthy elderly individuals and in those with underlying chronic disease. In our population, annual revaccination was associated with a significant mortality reduction among those aged 70 years and older. This result may reflect differences in age-related causes of death, which probably were less influenced by vaccination in those at a younger age than those in the highest age groups. Additionally, the observed lack of effect in the youngest age categories may be a result of a lower baseline risk of death. Interruption of yearly influenza vaccination was associated with a significantly increased mortality, but after restarting vaccination, mortality risk reduced again to a revaccination status level. Absence of protection from the vaccination may be an explanation for the observed risk increase, since individuals who interrupted vaccination for 2 or more consecutive years had a further increase in mortality risk.

Although a protective association between mortality and revaccination status in elderly persons has been suggested previously, only 1 case-control study has examined this association. In this study, a previous vaccination significantly increased vaccine effectiveness in the next season [9]. However, the study was not population-based; approximately half of the individuals were institutionalized. Nichol et al [7, 19] and Hak et al [5] studied the effect of influenza vaccination on long-term outcomes but did not take revaccination status into account. Gross et al. [20] published a meta-analysis on mortality risk in 20 cohort studies. Based on the current study, the large variability in effects identified by Gross et al. might be explained by different revaccination states, variations in epidemic activity, and population characteristics. It has also been proposed that variability of revaccination efficacy might be due to antigenic differences among the vaccine and epidemic strains [26]. Our study did not find an effect of first vaccination, but past studies and our previous study in the same database found a significant protective effect [6].

Our study has several strengths. We were able to assess the overall annual and epidemic effectiveness of annual influenza vaccinations as well as the effect of individual revaccinations. The study was population-based and less subject to selection bias, information bias, and confounding. In the Dutch health care system, all individuals are designated their own GP, so selection bias is unlikely. Information bias may have occurred if the vaccination was not recorded. However, such misclassification would likely be random because exposure is prospectively recorded before death occurred. Such random misclassification would tend to reduce the size of the

estimate, suggesting that the real protective effect could be even greater. All-cause mortality was chosen as an end point because it is an important outcome, which cannot be misclassified. Deaths were unlikely to have been missed since death rates in IPCI were similar to national data on mortality. As discussed above, confounding by indication is possible but in this study, comorbidity and a higher risk of mortality would be an indication for vaccination, reducing the likelihood of confounding as an explanation for the observed effect. Moreover, perceived good health has, among others, been reported as a reason for non-compliance with the influenza vaccination program [27]. Indeed, compared with those refusing vaccination, mortality risk was not reduced following a first or revaccination. However, excluding those who refused vaccination from the reference category resulted in a significant adjusted risk reduction following a first and revaccination. In addition, we adjusted for chronic respiratory tract disease, cardiovascular disease, hypertension, diabetes mellitus, malignancies, and chronic renal insufficiency, either pre-existing or having developed during follow up, because they were both indications for vaccination and independent risk factors for mortality. Even if some residual confounding by indication cannot be excluded, a poorer prognosis in individuals who were vaccinated would mean that our results would tend to underestimate the protective effect of annual revaccination [25].

In summary, our study shows that annual revaccination against influenza in a population of community-dwelling elderly persons is associated with a reduction of mortality risk. This study supports the recommendation for yearly influenza vaccination for elderly individuals, not only for those with comorbid illness but also in those without comorbidity and in patients 80 years or older. Because influenza vaccination is inexpensive and safe, clinicians should recommend annual influenza revaccination for such patients.

References

1. Murray CJL, Lopez AD. *Mortality by cause for eight regions of the world: Global Burden of Disease Study*. Lancet 1997; 349: 1269-76
2. Sprenger MJW, Mulder PG, Beyer WE, van Strik R, Masurel N. *Impact of influenza on mortality in relation to age and underlying disease,* 1967-1989. Int J Epidemiol 1993, 22: 334-40
3. van Essen GA, Palache AM, Forleo E, Fedson DS. *Influenza vaccination in 2000: recommendations and vaccine use in 50 developed and rapidly developing countries*. Vaccine 2003, 21: 1780-1785
4. Govaert ThME, Thijs MCTMCN, Masurel N, Sprenger MJW, Dinant GJ, Knottnerus JA. *The efficacy of influenza vaccination in elderly individuals. A randomized double blind placebo controlled trial*. JAMA 1994, 272; 21: 1661-5
5. Hak E, Nordin J, Wei F, Mullooly J, Poblete S, Strikas R, Nichol KL. *Influence of high risk medical conditions on the effectiveness of influenza vaccination among elderly members of 3 large managed care organisations*. CID 2002, 35: 370-7
6. Voordouw ACG, van der Linden PD , Simonian S, van der Lei J, Sturkenboom MCJM, Stricker BHC. *Influenza vaccination in community dwelling elderly. Impact on mortality and influenza associated morbidity*. Arch Int Med 2003, 163: 1089-94
7. Nichol KL, Nordin J, Mullooly J, Lask R, Fillbrandt K, Iwane M. I*nfluenza vaccination and reduction in hospitalisations for cardiac disease and stroke among the elderly*. N Engl J Med 2003, 348: 1322-32
8. McElhaney JE, Meneilly GS, Beattie BL, et al. *The effect of influenza vaccination on IL2 production in healthy elderly: implications for current vaccination practice*. J Gerontol 1992, 47; 1: M3-8
9. Ahmed AH, Nicholson KG, Nguyen-van Tam JS. *Reduction in mortality associated with influenza vaccine during the 1989-1990 epidemic*. Lancet 1995, 346: 591-5
10. Beyer WEP, Palache AM, Sprenger MJW et al. *Effects of repeated annual influenza vaccination on vaccine seroresponse in young and elderly adults*. Vaccine 1996, 14; 14: 1331-9
11. de Bruijn IA, Remarque EJ, Beyer WE, le Cessie S, Masurel N, Ligthart GJ. *Annually repeated influenza vaccination improves humoral responses to several influenza strains in healthy elderly*. Vaccine 1997, 15, 1323-1329
12. Saah AJ, Neufeld R, Rodstein M et al. *Influenza vaccine and pneumonia mortality in a nursing home population*. Arch Int Med 1986, 146: 2353-7
13. Arden N, Monto AS, Ohmit SE. *Vaccine use and the risk of outbreaks in a sample of nursing homes during an influenza epidemic*. Am J Publ Health 1995, 85; 3: 399-401
14. Keitel WA, Cate TR, Couch RB. *Efficacy of sequential annual vaccination with inactivated influenza virus vaccine*. Am J Epidemiol 1988, 127; 2: 353-64
15. Keitel WA, Cate TR, Couch B, Huggins LL, Hess KR. *Efficacy of repeated annual immunization with inactivated influenza virus vaccines over a five year period*. Vaccine 1997, 15; 10: 1114-22
16. Hoskins TW, Davies JR, Smith AR, Miller CL, Allchin A. *Assessment of inactivated influenza vaccine after 3 outbreaks of influenza A at Christ's Hospital*. Lancet 1979, 1; 8106: 33-5
17. Beyer, WE, de Bruijn IA, Palache AM, Westendorp RG, Osterhaus AD. *Protection against influenza after annually repeated vaccination: a meta-analysis of serologic and field studies*. Arch Intern Med 1999, 159; 2: 182-8.
18. Carrat, F Valleron AJ, *Influenza mortality among the elderly in France, 1980-90: ho many deaths may have been avoided through vaccination*? J Epid Comm Health Care 1995, 49: 419-25

19. Nichol KL, Baken L, Nelson A. *Relation between influenza vaccination and outpatient visits, hospitalisation and mortality in elderly persons with chronic lung disease.* Ann Int Med 1999, 130: 397403
20. Gross PA, Hermogenes AW, Sacks HS, Lau J, Levandowski RA. *The efficacy of influenza vaccine in elderly persons. A meta-analysis and review of the literature.* Ann Int Med 1995, 123; 7: 518-27
21. Nichol KL, Margolis KL, Wuorenma, J, Von Sternerg T. *The efficacy and cost-effectiveness of vaccination against influenza among elderly persons living in the community.* N Engl J Med 1994, 31: 778-84
22. Lamberts H, Wood M. *The birth of the International Classification of Primary Care (ICPC). Serendipity at the border of Lac Leman.* Fam Pract 2002, 19; 5: 433-5.
23. Vlug AE, van der Lei J, Mosseveld BMTh, et al. *Postmarketing surveillance based on electronic patient records: The IPCI project.* Meth Inform Med 1999, 38: 339-44
24. de Jong JC, Beyer WE, Palache AM, Rimmelzwaan GF, Osterhaus AD M*ismatch between the 1997/1998 influenza vaccine and the major epidemic A(H3N2) virus strain as the cause of an inadequate vaccine induced antibody response to this strain in the elderly.* J Med Virol 2000, 61: 94-9
25. Smith DJ, Forrest S, Ackley DH, Perelson AS. *Variable efficacy of repeated annual influenza vaccination.* PNAS 1999, 96; 24: 14001-6
26. van Essen GA, Kuyvenhoven MM, de Melker RA. W*hy do healthy elderly people fail to comply with influenza vaccination?* Age Ageing. 1997, 26:275-9
27. de Jong JC– *Personal communication*

4

Annual influenza vaccination and risk of sudden cardiac death in community dwelling elderly persons

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Abstract

Context

Influenza infections are associated with an increased risk of acute cardiac events. An effect of influenza vaccination on the risk of sudden cardiac death (SCD) has been suggested, but has not been systematically studied.

Objective

We investigated the effect of a first influenza vaccination and revaccination on SCD in community dwelling elderly.

Design and setting

We performed a population-based cohort study using the computerized Integrated Primary Care Information (IPCI) database in the Netherlands in subjects aged 65 years or older during the period 1996 through 2002. For each year, the cumulative exposure to influenza vaccination since study entry was computed.

Population

Community dwelling elderly aged 65 years or older on January 1st of the year of study entry.

Main outcome measure

The risk of SCD after a first vaccination or a revaccination was compared to no vaccination using a time-varying multivariate Cox-proportional hazard model, adjusted for age, gender, smoking and underlying chronic disease.

Results

The study population comprised 23,977 persons in whom we identified 267 cases of sudden cardiac death. Overall, any influenza vaccination or revaccination was associated with a non-significant risk reduction of SCD. A significant 2-3 times higher risk of SCD following a first vaccination was observed, in the subjects without co-morbidity and those with baseline age<70 years. In comparison to a first vaccination, revaccination was associated with a hazard ratio of 0.53 ($CI_{95\%}$: 0.36-0.77).

Conclusions

This study supports a protective benefit of influenza revaccination on the risk of sudden cardiac death in a population of community dwelling elderly. The observed increased risk following a first vaccination requires further study.

Introduction

Cardiovascular morbidity and mortality, particularly acute myocardial infarction, stroke and sudden cardiac death show seasonal variation, with peak incidences during winter months [1-5]. Clinicians have long noticed that approximately 30% of myocardial infarctions are preceded by an upper respiratory tract infection and influenza activity has been suggested as an explanation for the winter peak of myocardial infarctions [6]. Sudden Cardiac Death (SCD) has been suggested as a possible complication following influenza virus infection [2]. Influenza virus has been isolated from myocardial tissues of individuals who died from SCD and SCD cases were found to have a significantly higher proportion of influenza infection than did matched controls [7, 8].

In line with these observations, a significantly decreased risk of all cause mortality, death from cardiovascular causes and cardiovascular hospitalisations has been reported following influenza vaccination [1, 4, 9-11] . Given the increased risk of SCD following an influenza infection, and the decreased mortality following influenza vaccination it was hypothesized that influenza vaccination may also protect against SCD.

To investigate the association between influenza vaccination and the risk of sudden cardiac death we performed a cohort study in community dwelling elderly aged 65 years or older during the epidemic influenza seasons covering 1996-2002.

Methods

Setting

In the Netherlands, a nationwide influenza vaccination program has been active since 1997. The vaccination program is executed by GPs during annual mass vaccination days in October and November, during which all individuals aged 65 years and older and adults and children with predefined high risk factors are invited to participate in the vaccination campaign free of charge. General practitioners (GPs) register the vaccination in the electronic patient record.

All data for this study were retrieved from the Integrated Primary Care Information (IPCI) project, a longitudinal observational database, containing data from computer-based medical patient records from a group of 150 GPs in the Netherlands. In the Dutch health care system, the GP has a pivotal role by acting as a gatekeeper for all medical care. Nearly every citizen is registered with a GP. Details of the database have been described elsewhere [12, 13]. Briefly, the database contains the complete

medical records on approximately 530,000 citizens. The electronic records contain anonymous data on demographics, symptoms (in free text), diagnoses (using the International Classification for Primary Care and free text) from GPs and specialists, referrals, laboratory findings, hospitalisations, and drug prescriptions, including their indications and dosage regimen. To maximise completeness of the data, GPs participating in the IPCI project are not allowed to maintain a system of paper-based records besides the electronic medical records. The IPCI project complies with European Union guidelines on the use of medical data for medical research and has been proven valid for pharmaco-epidemiological research in several validation studies that evaluated the quality of the available information [12]. The Scientific and Ethical Advisory Board of the IPCI project approved this study.

Source population

The source population comprised all subjects of 65 years and older, who were registered with a GP who participated in the IPCI project for at least 1 year and who registered vaccinations in the electronic patient record. GPs with inconsistent registration, defined as a vaccination coverage of <25% or a variation of the coverage rate of ≥50% over the years, were excluded. The study period started on October 1, 1996 and ended on September 30, 2002. All subjects were followed until death, transferral out of practice, date of last data draw down or end of the study period, whichever came first. Individuals with a history of malignancies were excluded.

Exposure definition

The exposure of interest was influenza vaccination. The cumulative number of influenza vaccinations was determined between October 1st and December 31st of each calendar year. Exposure status was categorized into 3 mutually exclusive categories including non-exposed (non-vaccinated or vaccination refusal), 1st vaccination, and revaccination (any subsequent vaccination). A first vaccination status was defined as a first vaccination after study entry with no recorded influenza vaccination prior to study entry. Persons who were vaccinated prior to study entry were classified as being revaccinated. When a vaccination series was interrupted, it was categorised as such. Finally, restart after one or more years of interruption was also categorised separately. Once in the interruption category, subjects stayed in it until vaccination restart and vice versa. Consequently, in this time-varying approach of exposure, individuals contributed information to different exposure categories during follow-up.

Case definition

The computerized medical and demographic data were screened for deaths that occurred during the study period. The medical records of identified cases of death were reviewed manually to assess whether death could be classified as sudden cardiac death. Validation was performed independently by two physicians who were blinded to exposure (SMJMS, GSB) and in case of discrepancy, a third expert (BHChS) arbitrated. Case assessment was based on the most recent definition of sudden cardiac death [14, 15]. Cases were classified as (probable) sudden cardiac death if the medical record indicated that death occurred within one hour after the onset of cardiovascular symptoms and if the following wording was found in the free text: "sudden cardiac death", "acute cardiac death", "mors subita", "sudden death", "died suddenly", "died unexpectedly", or if this was an unwitnessed, unexpected death of someone seen in "good health" or in a stable medical condition less than 24 hours previously and without evidence of a non-cardiac cause (e.g., pneumonia, convulsion, choking or stroke). Suicides were excluded.

Deaths were allocated to the most recent vaccination status.

Co-variates and risk factors

Known risk factors for sudden cardiac death and other co-variates were gathered from the medical records through computerised searches and manual assessment. The co-variates that were evaluated included age, gender, smoking, chronic respiratory tract disease (chronic obstructive pulmonary disease, emphysema, chronic bronchitis, asthma); chronic cardiovascular disease (heart failure, angina pectoris, status after myocardial infarction or cerebro-vascular accident, aortic aneurysm, chronic arterial dysfunction), hypertension, diabetes mellitus and chronic renal insufficiency.

The presence of these conditions at study entry or their development at any time during follow-up was included in the analysis. The population without co-morbidity were those who had no co-morbidity at baseline and did not develop any of the predefined conditions during follow-up. Information on the size and timing of each influenza epidemic was obtained from Dr. JC de Jong of the National Influenza Center, Erasmus MC Rotterdam.

Statistical analysis

The association between influenza vaccination and sudden cardiac death was estimated by using multivariate time varying Cox proportional hazard models. To fully adjust for the strong influence of age on death, we used age in days as time axis. The

exposure status of a subject on the date of the sudden cardiac death was compared to all subjects in the cohort, who at that moment in follow-up had exactly the same age as the subject who died.

Over the complete follow up period, we investigated the effect of vaccine exposure on the risk of sudden cardiac death in 3 analyses: firstly, any vaccination (1st or revaccination) versus no vaccination. Secondly the effects of a first vaccination or revaccination versus no previous vaccination were assessed and finally any revaccination versus a first vaccination.

Co-variates that were univariately associated with SCD (at a $p<0.1$ level) were included in the models. We investigated potential effect modification of the presence of co-morbidity, and age.

Subsequently, we performed several sub-analyses related to the time and to vaccine refusers. First, we compared the risk of sudden cardiac death during the epidemic season (defined as the first day of the first week of the recorded epidemic until the last days of the last week of the recorded epidemic (details were reported elsewhere) [9]. Second, we studied the association between influenza vaccination and sudden cardiac death in the period between the individuals' vaccination date and the start of the epidemic (pre-epidemic season). Finally, we investigated the risk of sudden cardiac death in the remaining period between the last day of the epidemic season until the individuals' vaccination date of the next year (post-epidemic period). We also investigated whether the effect of vaccination on the risk of SCD differed between vaccine refusers and other non-users.

All analyses were performed with SAS software, version 8.2 using the procedure Proc Phreg.

Results

In the study population of 23,977 persons, we identified 267 cases of sudden cardiac death. The mean age at baseline was 73.0 years and 41% of the population was male. Cases of SCD had a mean age at baseline of 75.9 years and approximately 51% was male (table 1). Known potential risk factors for SCD, notably chronic cardiovascular disease, chronic respiratory disease, hypertension and diabetes mellitus were all associated with an increased risk (table 1).

In the overall population, influenza vaccination was associated with a non-significant 12% reduction in SCD risk which increased to a non-significant 21% following revaccination (table 2). A first vaccination was associated with a non-significant 50% increase in SCD risk (HR, 1.50; $CI_{95\%}$: 0.98-2.32). Compared to a first vaccination the risk of SCD following revaccination was 0.53 ($CI_{95\%}$: 0.37-0.77).

Table 1: Characteristics of the study population

Variable	No. (%) Population characteristics at study entry (n=23977)		SCD at follow up (n=267)		Risk of SCD during follow up, univariate HR (± CI_{95})
Gender					
Female	14101	(58.8)	131	(49.1)	Reference
Male	9876	(41.2)	136	(50.9)	1.74 (1.39-2.22)
Baseline age, y					
65-69	9764	(40.7)	53	(19.8)	Reference
70-79	9583	(40.0)	131	(49.1)	2.52 (1.83-3.46)
≥ 80	4630	(19.3)	83	(31.1)	3.30 (2.34-4.65)
Co-morbidities					
No co-morbidity	11711	(48.8)	52	(19.5)	Reference
Co-morbidity #	12266	(51.2)	215	(80.5)	2.07 (1.53-2.81)
Cardiovascular disease	5553	(23.2)	161	(60.3)	1.98 (1.54-2.54)
Hypertension	5860	(24.4)	86	(32.2)	1.09 (0.84-1.43)
Diabetes mellitus	2729	(11.4)	68	(25.5)	2.15 (1.60-2.87)
Chronic respiratory disease	3124	(13.0)	56	(21.0)	1.31 (0.94-1.82)
Renal insufficiencies	193	(0.8)	8	(3.0)	3.11 (1.38-6.98)
Life style					
Smoking	4824	(20.2)	48	(18.0)	1.44 (1.02-2.03)

Abbreviations: CI, confidence interval; HR: hazard ratio

(#) During follow up another 4341 individuals developed one or more co-morbidities, resulting in a total of 14607 individuals with any form of co-morbidity anytime during the study.

Table 2: Adjusted hazard ratios (*) of sudden cardiac death in the overall population

	Reference group: persons not previously vaccinated		
Influenza vaccination	**cases**	**Crude RR**	**Adjusted HR(±$CI_{95\%}$)**
Any vaccination	179	1.02	0.88 (0.64-1.20)
First vaccination	34	*1.59*	1.50 (0.98-2.32)
Revaccination	145	0.94	0.79 (0.58-1.10)
		Reference group: first vaccination	
Revaccination		*0.59*	*0.53 (0.36-0.77)*

Abbreviations: CI, confidence interval; HR, hazard ratio.

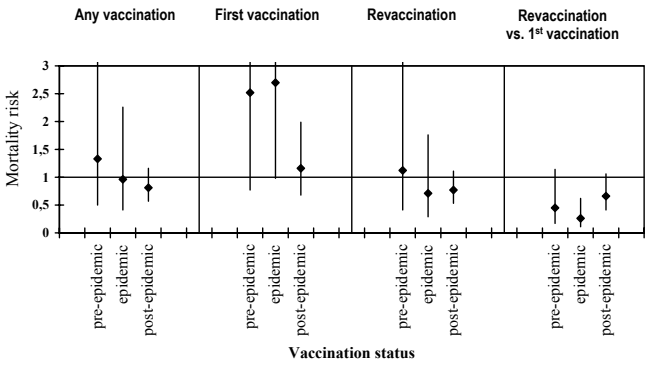
(*): adjustment for gender, underlying chronic diseases (cardiovascular diseases, respiratory diseases, hypertension, renal insufficiency, diabetes mellitus, and smoking

In the population with comorbidity, any vaccination as well as any revaccination was associated with a non-significantly reduced risk of SCD as compared to non-use. In the population without comorbidity, a revaccination resulted in a non-significant 32% reduction, but a first vaccination was associated with significantly increased risk of SCD (HR, 2.49, $CI_{95\%}$:1.17-5.29).

Higher baseline age appeared to be associated with a tendency towards a stronger beneficial effect of any influenza vaccination or revaccination but the differences with younger age-groups were non-significant.

The influence of the influenza epidemic on the association between vaccination and SCD is summarised in figure 1. Both during the pre-epidemic and the epidemic season we observed an increased, though non-significant risk of SCD following a first vaccination. Following revaccination the risk was lowest during the epidemic period, but none of these outcomes was significant. In the epidemic period, however, the risk reduction observed for any revaccination compared to a first vaccination was significant. In the post-epidemic time period, all point estimates were below 1 but none reached statistical significance.

Figure. Adjusted hazard ratios (*) for sudden cardiac death by time period, stratified by vaccination
Abbreviations: CI, confidence interval; HR, hazard ratio.



(*) adjustment for gender, underlying chronic diseases (cardiovascular diseases, respiratory diseases, hypertension, renal insufficiency, diabetes mellitus, and smoking

X-axis expresses the different periods:

Pre-epidemic = time between index date and start of the epidemic

Epidemic = time between first day of the first week of the epidemic until the last day of the last week of the epidemic

Post-epidemic = time between last day of the last week of the epidemic until the next index date.

Y-axis: expresses the hazard ratio for mortality risk following vaccination versus no previous vaccination (excluding vaccine refusers) ± 95% confidence intervals

Table 3: Adjusted hazard ratios (*) of sudden cardiac death, stratified by comorbidity and age.

Co-morbidity	Present		Absent	
	Reference group: no previous vaccination			
	cases	adjusted HR(±$CI_{95\%}$)	cases	adjusted HR(±$CI_{95\%}$)
Any vaccination	149	0.83 (0.57-1.20)	30	0.94 (0.52-1.72)
First vaccination	24	1.20 (0.71-2.04)	10	*2.49 (1.17-5.29)*
Revaccination	125	0.78 (0.54-1.14)	20	0.68 (0.35-1.32)
	Reference group: first vaccination			
Revaccination		0.65 (0.41-1.03)		*0.27 (0.13-0.56)*

Baseline age	<70 years		70-79 years		≥ 80 years	
	Reference group: no previous vaccination					
	cases	adjusted HR(±$CI_{95\%}$)	cases	adjusted HR(±$CI_{95\%}$)	cases	adjusted HR(±$CI_{95\%}$)
Any vaccination	34	1.27 (0.60-2.67)	91	0.83 (0.52-1.30)	54	0.55 (0.29-1.07)
First vaccination	8	*2.73 (1.10-6.75)*	17	1.31 (0.69-2.50)	9	1.16 (0.52- 2.56)
Revaccination	26	1.02 (0.47-2.20)	74	0.76 (0.48-1.22)	45	0.70 (0.41-1.22)
	Reference group: first vaccination					
Revaccination		*0.37 (0.18-0.80)*		0.58 (0.33-1.02)		0.61 (0.30-.1.25)

Abbreviations: CI, confidence interval; HR, hazard ratio.

(*) adjustment for gender, underlying chronic diseases (cardiovascular diseases, respiratory diseases, hypertension, renal insufficiency, diabetes mellitus, and smoking

Discussion

The results of our study indicate that influenza revaccination, but not a first vaccination may be associated with a reduced risk of sudden cardiac death. In subjects without comorbidity and those younger than 70 years at baseline, an increased risk of SCD was seen after a first vaccination.

We have no biological explanation for the finding of an increased risk after first vaccination, especially as it seemed confined to individuals without co-morbidity and those at younger age. The number of cases in this subgroup, however, was small and precision was low. A trend towards an increased risk was also observed during the pre-epidemic and epidemic period, i.e. the winter period where respiratory infections, including influenza infections may contribute to cardiovascular morbidity and mortality. Recently it was shown that acute infections are associated with a transient increase in the risk of vascular events [16]. However, influenza vaccination was associated with a decreased risk during the first 4 weeks postvaccination. It is not clear whether in that study these were first or revaccinations [16]. In our study 6 of the 32 persons who died from SCD in the pre-epidemic period died following a first vaccination: one patient after 5 day; the other 5 between 17 and 72 days after vaccination.

Following revaccination the observed relative risks all showed a protective effect. As these risks were measured against non-vaccinated controls, one must assume that the observed effect is a consequence of vaccine effectiveness. Also previous studies found a reduced incidence of acute ischemic events and death following influenza vaccination [9-11, 17], although vaccination was not associated with a reduced risk of recurrent coronary events [18]. In one small case control study influenza vaccination was associated with a 49% risk reduction of primary cardiac arrest [19]. In this study, however, vaccination exposure was assessed by spouse reports, which may have resulted in information bias. The observed lack of statistical significance in our study might be a result of insufficient power.

In our population, we were able to take advantage of the fact that in the Dutch health care system all medical information (including specialist and hospital care) is collected at practices that cover the general population instead of selected socio-economic groups. As a consequence, there was extensive information available on drug use, potential confounders and the circumstances around death. As all medical data on all population members is available, selection bias was unlikely. Another advantage of our study is that we were able to distinguish between first vaccinations and revaccinations. Previous studies addressing morbidity and mortality outcomes have given variable results, possibly because of variable vaccination histories of the study subjects.

Nevertheless, also our study has potential limitations. Although, we cannot exclude that some misclassification of outcome occurred, this is probably minimal since general practitioners consistently register deaths. Also the incidence of SCD was comparable to that of other sources. Misclassification of influenza vaccination is possible, but will be low and non-differential, since all data were recorded prospectively in the database and vaccination is supplied almost exclusively by the GP [9, 10].

Confounding by indication may have occurred and explain why we found an increased risk of SCD in the group of relatively young elderly without co-morbidity who received a first vaccination. It is possible that in this small group of cases the first vaccination occurred because of a recently recognized indication which was not yet notified in the GP-records. Even if residual confounding by indication also occurred in the other subgroups, we will have underestimated rather than overestimated the true effect of influenza vaccination on SCD. Moreover, in a direct comparison of revaccination to first vaccination which both have the same indication, revaccination was associated with a statistically significant risk reduction of SCD. Other potential sources for confounding were unlikely as we adjusted for all known factors to be associated with an increased risk of influenza associated morbidity and mortality and factors associated with an increased risk of SCD. In a subanalysis, those refusing vaccination with baseline age< 70 years were at a significantly lower risk of SCD

than the remaining non-vaccinated control group. However, this was not observed for subjects without co-morbidity. The belief to be in good health has been reported as reason for vaccination refusal [20]. A lower risk for those refusing vaccination was also observed in a previous study by our group [9].

In conclusion, we found an unexplained risk increase of SCD associated with a first influenza vaccination in a small subgroup of patients. In the remainder, revaccination was associated with a reduced risk of SCD. The results of this study support the benefit of annual influenza vaccination in reducing the risk of sudden cardiac death.

References

1. Armstrong, B.G., et al., *Effect of influenza vaccination on excess deaths occurring during periods of high circulation of influenza: cohort study in elderly people*, *BMJ*. 2004. p. 660.
2. Meyers, D.G., *Could influenza vaccination prevent myocardial infarction, stroke and sudden cardiac death? Am J Cardiovasc Drugs*. 2003. p. 241-4.
3. Naghavi, M., et al., *Influenza infection exerts prominent inflammatory and thrombotic effects on the atherosclerotic plaques of apolipoprotein E-deficient mice*, in *Circulation*. 2003. p. 762-8.
4. Nichol, K.L., et al., *Influenza vaccination and reduction in hospitalizations for cardiac disease and stroke among the elderly*, in *N Engl J Med*. 2003. p. 1322-32.
5. Stewart, S., et al., *Heart failure in a cold climate. Seasonal variation in heart failure-related morbidity and mortality*, in *J Am Coll Cardiol*. 2002. p. 760-6.
6. Madjid, M., et al., *Influenza and cardiovascular disease: a new opportunity for prevention and the need for further studies*, in *Circulation*. 2003. p. 2730-6. Epub 2003 Nov 10.
7. Drescher, J., et al., *Recent influenza virus A infections in forensic cases of sudden unexplained death*, in *Arch Virol*. 1987. p. 63-76.
8. Engblom, E., et al., *Fatal influenza A myocarditis with isolation of virus from the myocardium*, in *Acta Med Scand*. 1983. p. 75-8.
9. Voordouw, A.C., et al., *Annual revaccination against influenza and mortality risk in community-dwelling elderly persons*, in *JAMA*. 2004. p. 2089-95.
10. Voordouw, B.C., et al., *Influenza vaccination in community-dwelling elderly: impact on mortality and influenza-associated morbidity*, in *Arch Intern Med*. 2003. p. 1089-94.
11. Naghavi, M., et al., *Association of influenza vaccination and reduced risk of recurrent myocardial infarction*, in *Circulation*. 2000. p. 3039-45.
12. Vlug, A.E., et al., *Postmarketing surveillance based on electronic patient records: the IPCI project*, in *Methods Inf Med*. 1999. p. 339-44.
13. van der Lei, J., et al. *The introduction of computer-based patient records in The Netherlands*. in *Ann Intern Med*. 1993.
14. Myerburg RJ, *Cardiac arrest and sudden cardiac death*, in *Heart Disease, a text book of cardiovascular medicine*, E. Braunwald, Editor. 1997, WB Saunders Publishing Co.: New York. p. 742-79.
15. Priori, S.G., et al., *Task Force on Sudden Cardiac Death of the European Society of Cardiology*. Eur Heart J. Vol. 22. 2001. 1374-450.
16. Smeeth, L., et al., *Risk of myocardial infarction and stroke after acute infection or vaccination*, in *N Engl J Med*. 2004. p. 2611-8.
17. Gurfinkel, E.P., et al., *Influenza vaccine pilot study in acute coronary syndromes and planned percutaneous coronary interventions: the FLU Vaccination Acute Coronary Syndromes (FLUVACS) Study*, in *Circulation*. 2002. p. 2143-7.
18. Jackson, L.A., et al., *Influenza vaccination is not associated with a reduction in the risk of recurrent coronary events*
19. Siscovick, D.S., et al., eds. *Influenza vaccination and the risk of primary cardiac arrest*. Am J Epidemiol. Vol. 152. 2000. 674-7.
20. van Essen, G.A., M.M. Kuyvenhoven, and R.A. de Melker, *Why do healthy elderly people fail to comply with influenza vaccination?*, *Age Ageing*. 1997. p. 275-9.

5

Annual influenza vaccination in community dwelling elderly and the risk of lower respiratory tract infections or pneumonia

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Abstract

Context

Influenza vaccination has been associated with a reduction in hospitalisation for respiratory conditions in elderly persons. Little is known, however, about the effect of influenza vaccination on the whole severity range of respiratory tract infections.

Objective

We investigated the effect of annual influenza vaccination on the occurrence of lower respiratory tract infections (LRTI) in community dwelling elderly.

Design and setting

We performed a population-based cohort study using the computerized Integrated Primary Care Information (IPCI) database in the Netherlands in subjects aged 65 years or older between 1996 and 2002. For each year, the individual cumulative exposure to influenza vaccination since study entry was computed.

Study population

Community dwelling elderly aged 65 years or older on January 1st of the year of study entry.

Main outcome measure

We compared the risk of LRTI after a first vaccination or revaccination with no vaccination using a time-varying multivariate Cox-proportional hazard model, adjusted for age, gender, smoking and underlying disease.

Results

In the study population of 26,071 subjects, 3,412 developed LRTI during follow-up. A first vaccination did not reduce LRTI risk. During epidemic periods revaccination reduced LRTI risk by 33% (95% CI: 8-52%) in individuals without comorbidity.

Conclusions

In this study, annual influenza revaccination was associated with a reduction in LRTI in community dwelling elderly.

Introduction

During winter periods, up to 30% of elderly experience acute respiratory infections of which up to 20% is attributed to influenza virus [1- 4]. Respiratory complications following infection with influenza virus include acute bronchitis, pneumonia and exacerbations of chronic bronchitis or asthma [5]. Accordingly, rates of hospitalisation for pneumonia and influenza, acute bronchitis and chronic respiratory disease are significantly higher during influenza periods [6, 7]. Acute bronchitis is the most common complication following influenza infection seen in primary care. The risk increases with age and in the presence of underlying conditions [8].

In one meta-analysis covering 9 cohort studies (including 6 in nursing homes) influenza vaccination reduced the risk of hospitalisation for pneumonia on average by 53%, but with a considerable range [9-11]. In a more recent meta-analysis in community dwelling elderly, the risk reduction of hospitalisation for respiratory conditions after influenza vaccination was less than 40% [12]. Other recent cohort studies in elderly patients confirm the results from the meta-analyses [13, 14] but indicate strong effect modification by risk profile [14-17].

So far, studies only addressed LRTI leading to hospital admission, whereas most LRTIs are dealt with in primary care. To investigate to what extent annual influenza vaccination is associated with the risk to develop hospitalized and non-hospitalized LRTI in community dwelling elderly, we conducted a population-based cohort study.

Methods

Setting

In the Dutch healthcare system, all persons have their own general practitioner (GP) who files all relevant medical details on their patients from primary care visits, hospital admissions and visits to outpatient clinics. Since 1997, GPs execute a nationwide influenza vaccination program for which they are reimbursed. During annual mass vaccination days in October and November, all individuals with predefined risk factors and those aged 65 years or older are invited to participate in the vaccination campaign free of charge. GPs register the vaccination in their patient records.

Data for this study were derived from the Integrated Primary Care Information (IPCI) database at the Department of Medical Informatics of the Erasmus Medical Center in Rotterdam, the Netherlands. The IPCI database is a general practice research database that contains electronic patient records on a cumulative popula-

tion of 530,000 patients from approximately 150 GPs. The information includes all medical data including demographic information, patient complaints and symptoms, diagnoses, results of laboratory tests, referral notes from consultants, hospital admissions and prescriptions. Prescriptions include drug name, Anatomical Therapeutical Chemical code, dosage form, dose, prescribed quantity, and indication. Symptoms and diagnoses are recorded using the International Classification for Primary Care as the coding system [18] but also as free text. As IPCI is the sole repository of medical records, the participating GP's do not keep additional paper records. To warrant anonymity, patient and practice identifiers are altered. The system complies with EU guidelines on the use of data for medical research and has shown to be valid for pharmacoepidemiologic research [19-21]. The IPCI internal review board approved the study.

Study population

For this study we selected all persons of 65 years or older with a permanent status in one of the IPCI GP practices. Eligible persons had at least one year of recorded database history which was required to adequately determine prior health status and vaccination status. To restrict misclassification of exposure we excluded all practices that did not consistently register influenza vaccination over the study years. Inconsistent registration included recorded vaccination coverage rates of less than 25% or a variation of ≥50% over the years. In the remaining population, we conducted a cohort study including the period between October 1, 1996 and September 30, 2002. The end of follow-up was defined as the first episode of LRTI, death, moving out of the GP practice or end of the study period, whichever came first.

Exposure definition

The cumulative number of influenza vaccinations since study entry was determined between October 1st and December 31st of each calendar year. Exposure was categorized into 9 mutually exclusive categories including non-exposed, 1st, 2nd, 3rd, 4th, 5th and 6th or 7th vaccination, vaccination interruption, and restart. Subjects without a recording of vaccination in their history or during follow up were considered as non-exposed until their first recorded vaccination. Non-exposed subjects could be flagged as vaccine refusers in the GP database. These persons were not automatically invited for the annual vaccination, until the moment that the individual indicated an annual invitation was appreciated. Persons with a vaccination history prior to study entry, started with the number of previously recorded vaccinations. Upon each additional consecutive vaccination during the study period, the cumula-

tive number increased by one. Once in the interruption category subjects stayed in it until vaccination restart as described previously [21]. In this time-varying approach of exposure analysis, individuals may contribute information to multiple exposure categories during follow-up.

Outcome definition

The primary outcome in this study was a first episode of LRTI, which was defined as pneumonia, acute bronchitis or an exacerbation of chronic bronchitis. LRTI was identified from the medical chart of the patient. LRTI were considered only as outcome if they resulted in antibiotic therapy or were confirmed by radiography and/or microbiology. To be able to compare our results to other studies we also assessed hospitalizations for pneumonia as secondary outcome. Events were allocated to the vaccination status defined in the preceding vaccination period.

Co-variates

Age, gender, smoking, antibiotic use and the number of GP visits were considered as co-variates. Furthermore, we identified seven disease clusters as potential confounders: chronic respiratory tract disease (chronic obstructive pulmonary disease, emphysema, chronic bronchitis, asthma), chronic cardiovascular disease (heart failure, angina pectoris, history of myocardial infarction or stroke, aortic aneurysm, chronic arterial dysfunction), diabetes mellitus, hypertension, chronic renal insufficiency, malignancies and neurological or psychiatric disorders (parkinsonism, dementia, polyneuropathy, multiple sclerosis, epilepsy, alcoholism, depression, psychosis, schizophrenia, Ménière's disease). Presence of these conditions at study entry or their development during follow-up was retrieved from the medical charts through automatic screening and manual validation. The population without comorbidity had no comorbidity at baseline and did not develop any of the predefined conditions during follow-up. Information on the characteristics of each influenza season was obtained from Dr. JC de Jong of the National Influenza Center, Erasmus MC Rotterdam.

Analyses

For estimation of the univariate association between vaccination, co-variates and the development of LRTI or (hospitalized) pneumonia we used a Cox proportional hazards model. Multivariate time varying Cox proportional hazard models were developed to estimate the hazard ratios for different vaccination states while adjusting

for all other risk factors. For all analyses, age expressed in days was taken as the time axis to fully adjust for age.

The effect of vaccine exposure on the risk of the outcome was evaluated for a first vaccination or revaccination versus no vaccination. Subsequently, the outcomes were stratified for presence of comorbidity and age at study entry (65-69 years, 70-79 years or >79 years). We first investigated these effects during the full follow-up period and subsequently during the epidemic period. The epidemic period started on the first day of the first week of the recorded epidemic and ended on the last day of the last week of the recorded epidemic. Hence the duration of the period varied per year. As a comparison, the association of influenza vaccination and LTRI was assessed also in the summer period (June, July and August) during which period there is almost no circulation of influenza virus in the Netherlands. In each analysis the reference group comprised all non-vaccinated subjects.

All results were expressed as hazard ratios with 95% confidence intervals ($CI_{95\%}$). For all analyses SAS software, version 8.2 using the procedure Proc Phreg was used.

Results

The study population comprised 26,071 elderly, of whom 58% was female (table 1). Mean (SD) age at study entry was 73.1 (7.4) years. In this population, 3,412 subjects developed a first episode of LRTI. In 1,295 patients this first LRTI episode was classified as pneumonia, and 455 of these patients were hospitalised with this condition. The risk of developing LRTI was higher in males, increased with age, was higher in the presence of comorbidity, and in smokers. Especially the presence of chronic respiratory diseases at baseline was associated with an increased risk of LRTI.

Vaccination coverage and epidemic characteristics of this population have been published previously [21]. In short, vaccination coverage varied between 64% and 74% and was slightly higher in the subpopulation with comorbidity (72% to 79%). Most vaccinations (96%) were given in October or November, the remainder in December. During the study, 59,111 influenza vaccinations were administered to 20,976 persons; 5,095 persons never received any influenza vaccination. All epidemics were predominated by A/H3N2 strains which showed a good match between circulating and vaccine strain(s) except for the 1997-1998 season (mismatch). Epidemic activity was mild to moderate in the 1996-1997 and 1999-2000 seasons, absent in the 2000-2001 season and mild in the other seasons. The peak activity of influenza-like illness ranged between 7 cases per 10,000 persons (2000-2001 season) and 32 cases per 10,000 persons per week (1999-2000 season) and was observed between weeks 2 and 13 (Personal communication Dr. JC de Jong).

Table 1: Demographic characteristics and univariate hazard ratios for any of the outcomes during follow up.

Variable	No. (%)				Hazard ratio (± $CI_{95\%}$)
	Population characteristics at study entry (n=26,071)		Lower respiratory infections at follow up (n=3,412)		
Sex					
Female	15131	(58.0)	1796	(52.6)	Reference
Male	10940	(42.0)	1616	(47.4)	1.40 (1.31-1.50)
Age, y (mean ±SD)	73.1 ±7.4		77.0 ± 7.5		
65-69	10490	(40.2)	956	(9.1)	Reference
70-79	10522	(40.4)	1478	(14.6)	1.69 (1.67-1.70)
≥ 80	5049	(19.4)	978	(19.4)	2.14 (2.11-2.16)
Smoking					
No	20704	(79.4)	2477	(12.0)	Reference
Yes	5367	(21.6)	935	(17.4)	1.53 (1.40-1.68)
Co-morbidity					
None at study entry	12173	(46.7)	1129	(9.3)	Reference
Comorbidity at study entry#	13898	(53.3)	2283	(16.4)	2.49 (2.25-2.75)
Diabetes mellitus	3000	(11.5)	418	(13.9)	1.12 (1.01-1.24)
Hypertension	6414	(24.6)	887	(13.8)	1.04 (0.97-1.13)
Chronic respiratory disease	3487	(13.3)	977	(28.0)	3.15 (2.93-3.39)
Cardiovascular disease	6099	(23.4)	1065	(17.5)	1.57 (1.46-1.69)
Cancer	1034	(4.0)	156	(15.1)	1.40 (1.19-1.64)
Renal dysfunction	221	(0.8)	35	(15.8)	1.41 (1.01-1.97)
Neurological disease	3200	(12.3)	471	(14.7)	1.33 (1.24-1.43)

Abbreviations: CI, confidence interval; HR, hazard ratio.

(#) During the follow-up period comorbidity developed in another 2903 individuals resulting in a total of 16701 subjects with comorbidity at baseline or any time during follow-up.

Table 2 summarizes the association between influenza vaccination and LRTI. If we considered the full follow-up period a first influenza vaccination or revaccination was not associated with a reduction in the risk of LRTI. If we restricted follow-up to the epidemic period, the risk of LTRI following any revaccination decreased by 33% but only in subjects without comorbidity (table 2). Influenza vaccination did not protect against LTRI in persons with co-morbidity nor with chronic respiratory disease. Stratification by age suggested a tendency towards lower hazards of LRTI following revaccination in elderly, but this was not seen for first vaccinations.

We were able to differentiate those refusing vaccination from the rest of the non-vaccinated control group (data not shown). Persons with underlying comorbidity listed as vaccine refusers were at lower risk of LRTI compared to the rest of the non-vaccinated persons with co-morbidity. Contrary, persons without comorbidity

Table 2: Association between influenza vaccination and lower respiratory tract infection during the period 1996-2002

	First vaccination		Revaccination	
	cases	HR(± $CI_{95\%}$)	cases	HR(± $CI_{95\%}$)
Full period				
Total population	314	1.03 (0.90-1.19)	2208	1.08 (0.98-1.18)
Epidemic period				
Total population	79	0.86 (0.71-1.05)	567	0.94 (0.83-1.06)
Without comorbidity	13	0.90 (0.56-1.45)	58	*0.67 (0.48-0.92)*
With co-morbidity	66	0.83 (0.66-1.04)	509	0.95 (0.82-1.10)
Chronic respiratory disease	27	0.77 (0.55-1.08)	269	0.94 (0.75-1.15)
Baseline age				
<70 years	20	0.91 (0.61-1.36)	162	1.27 (0.98-1.65)
70-79 years	29	0.82 (0.60-1.12)	239	0.85 (0.70-1.04)
≥80 years	30	0.89 (0.64-1.24)	166	0.83 (0.67-1.01)
Summer period				
Total population	52	1.41 (0.99-2.01)	347	1.18 (0.92-1.52)

Abbreviations: CI, confidence interval; HR, hazard ratio.

Note: adjustment for gender, underlying chronic diseases (cardiovascular diseases, respiratory diseases, hypertension, malignancies, renal insufficiency, diabetes mellitus, and neurological diseases), smoking, number of antibiotic prescriptions and number of GP visits.

Table 3: Association between influenza vaccination and pneumonia over the period 1996-2002

	First vaccination		Revaccination	
	cases	HR ± $CI_{95\%}$)	cases	HR ± $CI_{95\%}$)
Full period				
Total population	93	0.84 (0.65-1.07)	839	0.96 (0.82-1.11)
Epidemic period				
Total population	26	0.87 (0.55-1.36)	216	0.89 (0.67-1.18)
Hospitalised patients	3	*0.29 (0.10-0.96)*	70	0.82 (0.49-1.36)
Without comorbidity	4	0.55 (0.19-1.61)	23	*0.50 (0.27-0.93)*
With co-morbidity	22	0.95 (0.58-1.57)	193	0.97 (0.70-1.35)
Chronic respiratory disease	8	0.65 (0.27-1.57)	107	1.03 (0.61-1.73)
Baseline age				
<70 years	7	0.95 (0.38-2.35)	55	1,31 (0.72-2.40)
70-79 years	4	0.49 (0.18-1.30)	88	0.98 (0.60-1.60)
≥80 years	15	1.12 (0.60-2.08)	81	0.66 (0.43-1.02)
Summer period				
Total population	19	1.14 (0.65-2.00)	144	1.00 (0.69-1.47)

Abbreviations: CI, confidence interval; HR, hazard ratio.

Note: adjustment for gender, underlying chronic diseases (cardiovascular diseases, respiratory diseases, hypertension, malignancies, renal insufficiency, diabetes mellitus, and neurological diseases), smoking, number of antibiotic prescriptions and number of GP visits.

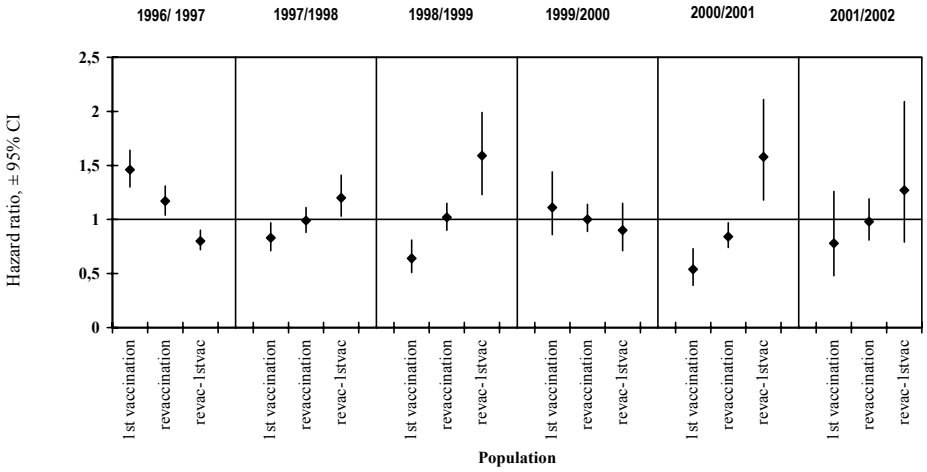
listed as vaccine refusers, were at a significantly higher risk of LRTI than the rest of the non-vaccinated control group without comorbidity.

Considering the full follow-up period, there was no benefit of first or revaccination on the risk of hospitalized and non-hospitalized pneumonia (table 3). During the epidemic period revaccination was associated with a 50% risk reduction of pneumonia, but only in the subpopulation without comorbidity. A first influenza vaccination was associated with a 71% risk reduction ($CI_{95\%}$: 4-90%) of hospitalized pneumonia, but no significant association was seen for revaccination.

Influenza vaccination was not associated with all LRTI or pneumonia during the summer period (tables 2, 3).

Only in the seasons with mild to moderate epidemic activity revaccination was associated with a reduced risk of LRTI compared to a first vaccination (Figure 1). During seasons with mild or no epidemic activity, subjects with an first vaccination consistently had a lower risk of LRTI than revaccinated subjects.

Figure 1. Association between influenza first or revaccination and lower respiratory tract infection during epidemic periods, stratified by year.



Note: adjustment for gender, underlying chronic diseases (cardiovascular diseases, respiratory diseases, hypertension, malignancies, renal insufficiency, diabetes mellitus, and neurological diseases), smoking, number of antibiotic prescriptions and number of GP visits.

X-axis: 1st vaccination, any revaccination, revaccination vs. first vaccination all fully adjusted.

Y-axis: hazard ratio indicates the risk of lower respiratory tract infection following first vaccination or revaccination vs. no vaccination, or following revaccination vs. first vaccination

Discussion

In the present study we showed that influenza vaccination reduces the risk of LRTI, but only in specific subgroups and during the epidemic period. The observed protection against hospitalization for pneumonia is in line with other studies [11-13]. In our study, revaccination was only beneficial in years with mild to moderate epidemic activity. This supports the necessity to analyse these morbidity outcomes by epidemic year. Others reported that the potential impact of influenza vaccination on the risk to develop pneumonia depends upon the individuals' risk profile [14, 16, 17]**.** With increasing age, a tendency towards a stronger effect of revaccination was noted in our study, although the estimates did not reach statistical significance. One explanation might be the gradual impairment of health status with age, resulting in a higher disease burden allowing for a stronger impact of vaccination. The tendency towards a reduced beneficial effect of a first vaccination, might reflect an impaired immune response in the elderly. Similar observations, although not distinguished between first vaccination and revaccination, led to the conclusion that the benefit of influenza vaccination in elderly was limited to those below 70 years [22].

Like all observational studies, also this study may suffer from selection bias, information bias and confounding. Selection bias was discarded as the data were obtained from computerized GP patient records that are population-based and independent of morbidity. Information bias may occur on exposure and outcome. Misclassification of the cumulative number of influenza vaccinations is possible but will be low since all data are recorded prospectively in the computer. The vaccination is supplied almost exclusively by the GP, and we only included practices with consistent vaccination coverage over the follow-up period. Misclassification of LRTI in a GP setting may be problem. To some extent, the validation process in free text is valuable to control for possible variations in the application of ICPC codes by GPs. However, to enhance sensitivity of the clinical criteria, microbiological confirmation is important [23]. Respiratory tract infections are caused by a variety of viruses and bacteria and are common in elderly, especially during winter months [23], but are often not confirmed [3]. Although influenza virus is the most commonly isolated specimen in respiratory infections in adults [24], other viruses (e.g. RSV, rhinoviruses, corona viruses) are more frequent in elderly [23]. Also clinical symptoms of LRTI can be misleading in community dwelling elderly [3]. Misclassification of LRTI may have been differential if GPs were less likely to diagnose LRTI in vaccinated persons in view of the assumed effectiveness.

Another potential problem is confounding by indication. Although the national recommendation was to vaccinate all elderly of 65 years and older, generally healthy individuals do not always follow such advice [25]. On the contrary, critically ill

patients may refuse vaccination or may not be offered the vaccination any more in view of their poor prognosis. However, this appeared not to be the case in our study. Persons with underlying comorbidity listed as vaccine refusers appeared to be in a relatively better health than the rest of the non-vaccinated control group, whereas those without comorbidity appeared to be relatively less healthy. Apparently, however, vaccine refusers do not have a consistent risk profile.

We suspected potential confounding by indication since pre-existing chronic respiratory tract disease, cardiovascular tract disease, diabetes mellitus, malignancy, chronic renal insufficiency and neurological disease were independent risk factors for increased influenza associated morbidity. Therefore, we adjusted for these risk factors. However, residual confounding is possible, and may have led to an underestimation of the true effect of influenza vaccination [23].

The strength of this study is that the effect of repeated vaccinations on LRTI or pneumonia could be estimated in each of 6 consecutive seasons in which epidemic and vaccine strain characteristics varied. The possible influence of vaccine strain selection on effectiveness of vaccination in repeat vaccinees has already been reported [26, 27]. Contrary to mortality, in the present study interruption and restart of the vaccination series had no obvious impact on vaccination effectiveness [21].

In summary, this study indicates that in a population of community dwelling elderly repeated influenza vaccination may reduce the risk to develop LRTI or pneumonia, in years with higher epidemic activity. Although the protective effect is modest influenza vaccination should be advised in view of the high background incidence of LRTI and because of the reduced mortality in elderly [21].

References

1. Falsey, A.R., et al., *Acute respiratory tract infection in daycare centers for older persons*. J Am Geriatr Soc, 1995. 43(1): p. 30-6.
2. Glathe, H. and W. Lange, *Influenza vaccination in older patients. Immunogenicity, epidemiology and available agents*. Drugs Aging, 1995. 6(5): p. 368-87.
3. Nicholson, K.G., et al., *Acute viral infections of upper respiratory tract in elderly people living in the community: comparative, prospective, population based study of disease burden*. BMJ, 1997. 315(7115): p. 1060-4.
4. Walsh, E.E., C. Cox, and A.R. Falsey, *Clinical features of influenza A virus infection in older hospitalized persons*. J Am Geriatr Soc, 2002. 50(9): p. 1498-503.
5. Nicholson, K.G., *Human Influenza*, in *Textbook of influenza*, H. A.J., Editor. 1998, reprint 2000, Blackwell sciences ltd.: London. p. 233-244.
6. McBean, A.M., J.D. Babish, and J.L. Warren, *The impact and cost of influenza in the elderly*. Arch Intern Med, 1993. 153(18): p. 2105-11.
7. Foster, D.A., et al., *Influenza vaccine effectiveness in preventing hospitalization for pneumonia in the elderly*. Am J Epidemiol, 1992. 136(3): p. 296-307.
8. Connolly, A.M., et al., *What are the complications of influenza and can they be prevented? Experience from the 1989 epidemic of H3N2 influenza A in general practice*. BMJ, 1993. 306(6890): p. 1452-4.
9. Gross, P.A., et al., *The efficacy of influenza vaccine in elderly persons. A meta-analysis and review of the literature*. Ann Intern Med, 1995. 123(7): p. 518-27.
10. Barker, W.H. and J.P. Mullooly, *Influenza vaccination of elderly persons. Reduction in pneumonia and influenza hospitalizations and deaths*. JAMA, 1980. 244(22): p. 2547-9.
11. Howells, C.H., et al., *Influenza vaccination and mortality from bronchopneumonia in the elderly*. Lancet, 1975. 1(7903): p. 381-3.
12. Vu, T., et al., *A meta-analysis of effectiveness of influenza vaccine in persons aged 65 years and over living in the community*. Vaccine, 2002. 20(13-14): p. 1831-6.
13. Nichol, K.L., *Complications of influenza and benefits of vaccination*. Vaccine, 1999. 17 Suppl 1: p. S47-52.
14. Hak, E., et al., *Influence of high-risk medical conditions on the effectiveness of influenza vaccination among elderly members of 3 large managed-care organizations*. Clin Infect Dis, 2002. 35(4): p. 370-7.
15. Nichol, K.L., *The additive benefits of influenza and pneumococcal vaccinations during influenza seasons among elderly persons with chronic lung disease*. Vaccine, 1999. 17 Suppl 1: p. S91-3.
16. Nichol, K.L., J. Wuorenma, and T. von Sternberg, *Benefits of influenza vaccination for low-, intermediate-, and high- risk senior citizens*. Arch Intern Med, 1998. 158(16): p. 1769-76.
17. Mullooly, J.P., et al., *Influenza vaccination programs for elderly persons: cost-effectiveness in a health maintenance organization*. Ann Intern Med, 1994. 121(12): p. 947-52.
18. Lamberts, H. and M. Wood, *The birth of the International Classification of Primary Care (ICPC). Serendipity at the border of Lac Leman*. Fam Pract, 2002. 19(5): p. 433-5.
19. Vlug A.E., et al., . *Postmarketing surveillance based on electronic patient records: The IPCI project*. Meth Inform Med, 1999(38): p. 339-44.
20. Voordouw, B.C., et al., *Influenza vaccination in community-dwelling elderly: impact on mortality and influenza-associated morbidity*. Arch Intern Med, 2003. 163(9): p. 1089-94.
21. Voordouw, A.C.G., et al., *Annual revaccination against influenza and mortality risk in community dwelling elderly persons*. JAMA, 2004. 292(17): p. 2089-95.
22. Govaert, T.M., et al., *The efficacy of influenza vaccination in elderly individuals. A randomized double-blind placebo-controlled trial*. JAMA, 1994. 272(21): p. 1661-5.

23. Widdicombe, J. and S. Kamath, *Acute cough in the elderly: aetiology, diagnosis and therapy*. Drugs Aging, 2004. 21(4): p. 243-58.
24. Gonzales, R., et al., *Principles of appropriate antibiotic use for treatment of uncomplicated acute bronchitis: background*. Ann Intern Med, 2001. 134(6): p. 521-9.
25. van Essen, G.A., M.M. Kuyvenhoven, and R.A. de Melker, *Why do healthy elderly people fail to comply with influenza vaccination?* Age Ageing, 1997. 26(4): p. 275-9.
26. Smith, D.J., et al., *Variable efficacy of repeated annual influenza vaccination*. Proc Natl Acad Sci U S A, 1999. 96(24): p. 14001-6.
27. Smith, D.J., et al., *Mapping of the antigenic and genetic evolution of influenza virus*. Science, 2004. 305: p. 371-376.

6

Evaluation of serological trials submitted for annual relicensure of influenza vaccines to regulatory authorities between 1992-2002

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Abstract

Introduction

As part of an annual relicensure requirement, marketing authorization holders (MAH) of trivalent inactivated influenza vaccines submit serological studies performed in healthy adults and elderly to the regulatory authorities of the European Union.

Objective

The objective of the present study was to analyse the serological data submitted to the Dutch Medicines Evaluation Board (MEB) between 1992 and 2002, with respect to their ability to assess the immunogenic properties of the vaccines.

Methodology

Serological data from immunogenicity trials for influenza vaccines submitted to the MEB as part of the annual relicensure dossier were analysed. This was done according to criteria described in the Committee of Human Medicinal Products Note for Guidance on harmonisation of requirements for influenza vaccines (CHMP NfG). These trials are typically uncontrolled and open label, and include 2 groups of at least 50 healthy adults and healthy elderly.

Results

In 48 age-defined trials, 4,518 persons (2,510 adults, 2,008 elderly) satisfied the CHMP pre-requirements. All but three trials fulfilled the CHMP criteria. However, in many studies pre-vaccination antibody levels were already high and very important for the fulfilment of the CHMP seroprotection criterion. Moreover, the serological response was age dependent. A history of previous influenza vaccinations significantly affected pre-, but not post-vaccination titres.

Conclusions

The CHMP criteria show serious methodological limitations, which affect their ability to identify influenza vaccines with low immunogenicity. The value and need of annual serological trials for relicensure influenza vaccines in the European Union may be questioned.

Introduction

Many countries recommend annual influenza vaccination for elderly and individuals with specified high risk conditions, with the objective to induce protection against influenza infection in the upcoming season [1]. Annually, the World Health Organisation issues an updated recommendation for the vaccine composition for the next season, based upon the expected circulating strains. As, in general, the strain composition changes each year, influenza vaccines are relicensed annually. Ideally, clinical vaccine efficacy is established in experimental field trials [2], but for an annual relicensure procedure, this approach is unrealistic.

Within the European Union, marketing authorization holders of influenza vaccines are required to provide clinical immunogenicity data to support the annual relicensure procedure, in addition to pre-clinical requirements addressing Good Manufacturing Practice, toxicity (e.g., endotoxin content), and antigenic content (i.e., potency as determined by the single radial immunodiffusion assay). The "Note for Guidance on Harmonisation of requirements for influenza vaccines" (CHMP/BWP/214/96) [3] describes the requirements and criteria for serological annual update trials. This procedure can only be followed if a first licensure, evaluating quality, immunogenicity and safety has been issued.

The underlying rationale of the CHMP-criteria is to assess the immunogenicity of annual influenza vaccines (in case of new components) according to pre-specified response parameters as a proxy for clinical protection. Although these parameters provide objective criteria, and are age specific they do not account for heterogeneity in response that may be due to other factors. For instance, indivuals are exposed, by infection or vaccination, to many influenza virus (sub) types and strains during their lifetime, which results in highly variable pre-vaccination titres [4]. Also, ageing and health status may affect humoral responses to influenza viral antigens [5-12], although immune function may be preserved in those reaching advanced age in overall good health [13]. These population characteristics can influence the haemagglutinin inhibiting (HI) antibody response induced by vaccination, independent of the vaccine. The influence of such vaccine-unrelated population characteristics on the seroprotection rate is unwanted. To control for these confounding factors, the relicensure trials use age stratification and a requirement of good general health of the trial population. Moreover, to address pre-vaccination titre, titre increase and response rate are used in addition to seroprotection rate. However, it is not clear to what extent these measures result in a more reliable assessment of the vaccines' immunogenicity [4].

The objective of this study was to describe response rates and to explore the influence of certain population characteristics (prevaccination titres, age and vaccination history) on postvaccination titres.

Methods

Data collection

We derived serological data from 51 immunogenicity trials for eight different trivalent inactivated split virus and subunit influenza vaccines that were submitted to the Dutch Medicines Evaluation Board (MEB) as clinical part of the annual relicensure dossier during the period 1992-1993 to 2002-2003. These data are stored on microfiche in the archives of the MEB.

The marketing authorisation holders of inactivated influenza vaccines in the Netherlands approved the use of their serological data for this study, provided that all product identifiers were removed and no direct between-product comparisons were published.

Inclusion criteria and CHMP criteria

According to the CHMP Note for Guidance on harmonisation of requirements for influenza vaccines [3], the immunogenicity of an influenza vaccine should be studied in at least 50 healthy adults aged 18 to 60 years and in at least 50 healthy elderly of 61 years or older. Blood samples are to be taken before and ~3 weeks after one dose and tested (in 2-fold) for anti-haemagglutinin antibody by either the haemagglutination inhibition (HI) assay or the single radial haemolysis (SRH) assay. For the HI assay, three statistics are measured: first, the proportion of subjects equal to or exceeding a post-vaccination titre of 40 (seroprotection rate); second, the mean geometric increase (also called mean fold increase, MFI), i.e. the geometric mean of the quotients of post- and pre-vaccination titres; and third, the combined proportion of seroconversions (i.e., previously seronegative subjects exceeding a post-vaccination titre of 40) and the proportion of subjects with a significant increase (i.e. that of previously seropositive subjects with a ≥4-fold increase in GMT). Together, these latter parameters are referred to as response rate. The CHMP NfG defines assessment criteria for these parameters. For adults seroprotection should be achieved postvaccination in at least 70% of the vaccinees, the other 2 parameters should be achieved in at least 40% of the vaccinees. In elderly, the corresponding percentages are 60%

and 30%. For each virus strain and each age class, at least one out of 3 criteria should be met.

Dataset

For inclusion in the final database documentation of the subjects' age, general health state and the complete set of pre- and post-vaccination titres was mandatory. We only regarded antibody titres determined by the HI assay, SRH results were excluded from the eligible population. From trials in adults, subjects <18 or >60 years-of-age were excluded, and from trials in elderly, subjects <61 years were excluded from the dataset. Trials, which covered both adult and elderly subjects, were stratified by age (either 18 to 60, or ≥61 years-of-age). A trial, which, after applying these selections, included less than 50 subjects, was removed.

Statistical methods

For database management and calculations, Microsoft® Excel 2002 and SPSS for Windows 10.0.1 1999 were used. Where appropriate, post-vaccination GMTs were adjusted for pre-vaccination GMTs by linear regression as described in Beyer et al. [4]. For comparisons between continuous sample statistics (e.g., GMT-values of subjects with negative versus positive vaccination history), the ratio was used as effect measure. For comparisons between binominal statistics (e.g., seroprotection rates), the absolute rate difference was used. Ratios throughout trials were combined by the inverse variance-weighted method [14], and rate differences by the meta-analysis method of DerSimonian and Laird for binominal data [15]. Interval estimates were given as 95% confidence intervals ($CI_{95\%}$).

Results

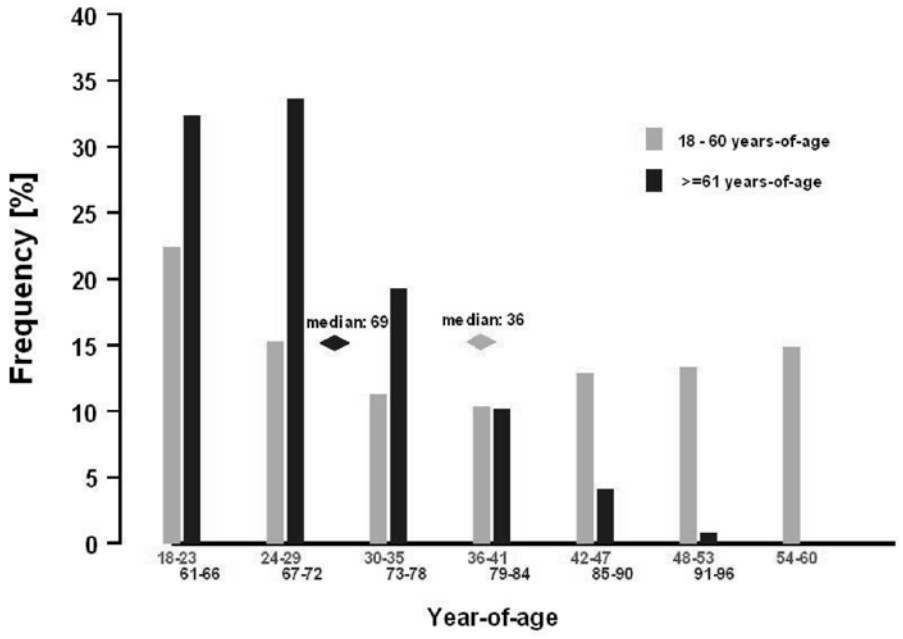
Fifty-one studies including 7,126 subjects were gathered from the archives. Three studies with 382 subjects using the SRH assay were excluded. A number of studies covered both age groups and were each separated into two (age-defined) trials (18-60, and >60 years). This resulted in a total of 71 strictly age-defined trials with 6,744 subjects. Twenty-three (23) trials including 2,226 subjects were excluded as they did not meet the inclusion criteria (see table 1). Of the remaining 4,518 vaccinees, gender was reported in 4,407 (55.5% female). Figure 1 shows the age distribution. The median age for adults was 36 and for elderly 69 year. Over calendar time the median age decreased both in elderly (from 71 in 1992 to 66 in 2002) as well as in

Table 1: Exclusion of trials and subjects to satisfy the CHMP pre-requisites

		No. trials	No. subjects
Originally retrieved data		71	6,744
Exclusions	(1) experimental vaccines[1]		12
	(2) no age reported	7	732
	(3)In adult studies: subjects <18 and >60 In elderly studies: subjects ≤ 60		39
	(4) no complete titre sets	1	275
	(5) reported co-morbidity		473
	(6) trial size <50 after exclusions	15	495
Final study population		48	4,518

([1]) Study arms with experimental adjuvant vaccine.

Figure 1. Age distribution for adult (18-60 years) and elderly (>60 years) study populations



adults (from 48 to 25 years) (figure 2). Before vaccination less than 40% of the adults and <21% of the elderly were seronegative for at least one of the strains (table 2). More than half of the elderly population was already seroprotected against one or more strains. In elderly the post-vaccination seroprotection criterion of 60% for the B-strain was reached almost entirely pre-vaccination (table 2).

Figure 2. Median age of the adult (18-60 years) and elderly (>60 years) study populations over the study period 1992 to 2002

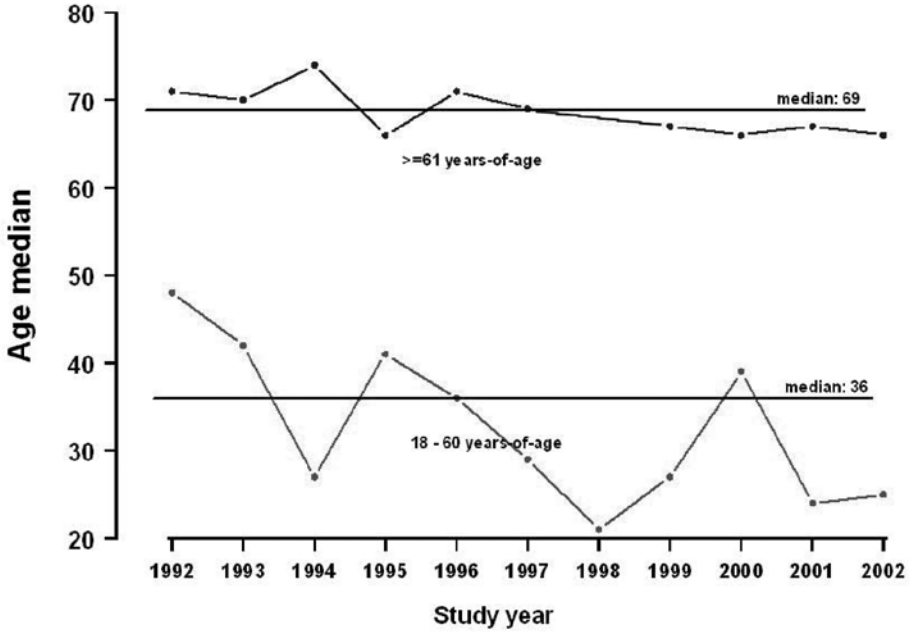


Table 2: Baseline serology

	No. (%)							
	Adults				Elderly			
Prevaccination titre	seronegative (<10)		seroprotected (≥40)		seronegative (<10)		seroprotected (≥40)	
A/H3N2	996	(39.7)	729	(29.0)	399	(19.9)	1066	(53.1)
A/H1N1	101	(40.4)	812	(32.4)	426	(21.2)	1019	(50.7)
B	646	(26.3)	1031	(42.0)	265	(13.5)	1166	(59.6)

Seronegative (<10) indicates HI titre< 10 before vaccination

Seroprotected (≥ 40) indicates HI titre before vaccination.

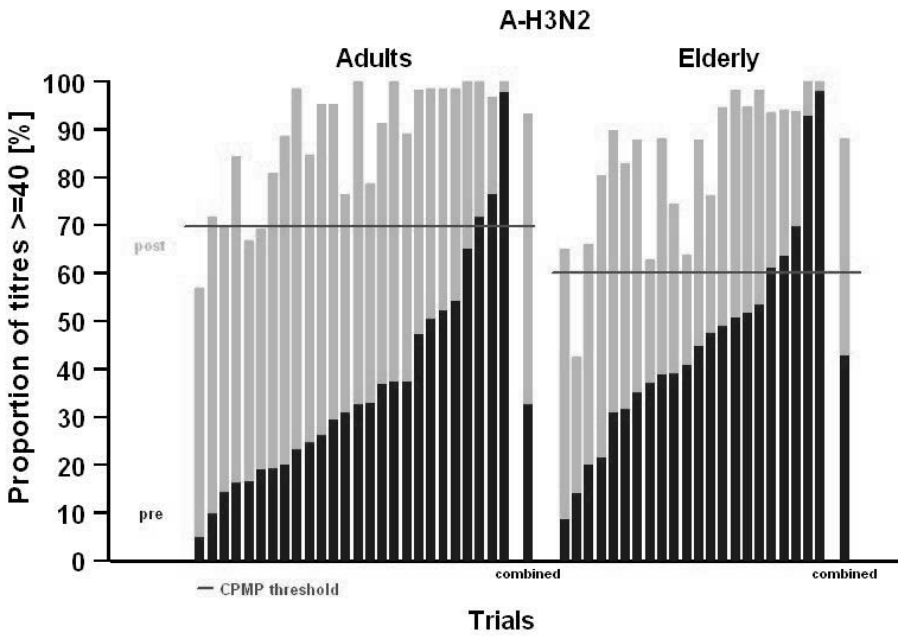
CHMP criteria in 48 age-defined trials

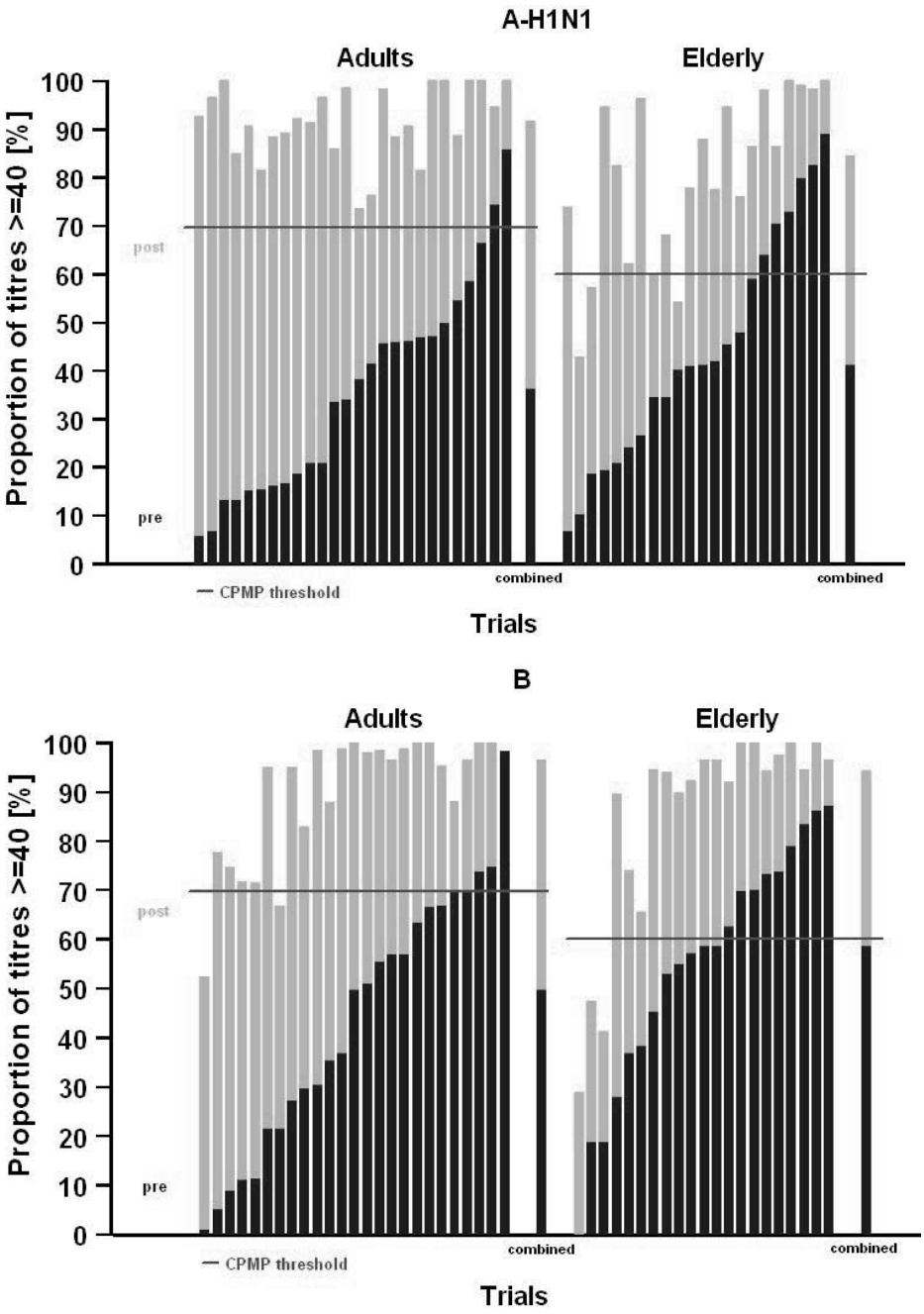
Table 3 presents the outcomes of the 48 trials. Single CHMP criteria were occasionally not met, most frequently the seroconversion criterion. Three times, a vaccine did not meet the CHMP criteria in the elderly, while it did in the corresponding adult trial: trial 32 (1994, corresponding adult trial: 8), trial 36 (1996, corresponding adult trial: 17), and trial 47 (1997, corresponding adult trial: 25).

The influence of pre-vaccination antibody titre on the seroresponse

In Figure 3a-c, pre- and post-vaccination seroprotection rates are shown for all individual trials, sorted by age class and pre-vaccination protection rates. A substantial number of trials had high prevaccination seroprotection rates. In some trials, prevaccination seroprotection rates exceeded the CHMP post-vaccination criterion. The trials, that failed to reach the post-vaccination threshold, had virtually all a low prevaccination GMT. The combined prevaccination seroprotection rates were higher in elderly than in adults (see also table 2), whereas the combined postvaccination

Figure 3a-c. Pre- and post-vaccination protection rates (defined as HI-titre≥40) for the individual trials and the combined ratio throughout the trials, stratified for age class (a) A-H3N2 strain, (b) A-H1N1 strain, and (c) B strain.





Pre = proportion of subjects reaching the seroprotection limit of 40 measured by HI-assay prevaccination.

Post= proportion of subjects reaching the seroprotection limit of 40 measured by HI-assay postvaccination.

CHMP threshold is defined as the proportion of seroprotected (titres>40) subjects postvaccination, for adults ≥70%, for elderly ≥ 60%.

Table 3: Compliance of the trials with the CHMP criteria

Age class	Trial	N	A/H3N2				A/H1N1				B			
			PR [%]	MFI	RR [%]	Req. met	PR [%]	MFI	RR [%]	Req. met	PR [%]	MFI	RR [%]	Req. met
CHMP criterion			**≥70%**	**≥ 2.5**	**≥40%**		**≥70%**	**≥ 2.5**	**≥40%**		**≥70%**	**≥ 2.5**	**≥40%**	
18-60 years	1	92	91,3	4,4	37,0[1]	Yes	85,9	10,6	63,0	Yes	52,2	5,2	43,5	Yes
	2	60	98,3	24,3	85,0	Yes	100,0	40,1	93,3	Yes	100,0	14,3	73,3	Yes
	3	67	98,5	14,4	74,6	Yes	92,5	82,7	74,6	Yes	74,6	8,2	41,8	Yes
	4	58	100,0	14,5	74,1	Yes	96,6	42,4	93,1	Yes	77,6	11,8	67,2	Yes
	5	233	84,5	9,6	68,7	Yes	90,6	6,6	60,1	Yes	95,3	5,6	66,1	Yes
	6	115	84,3	11,1	76,5	Yes	88,7	3,2	40,9	Yes	100,0	4,2	54,8	Yes
	7[2]	55	76,4	3,4	32,7	Yes	94,5	2,3	20,0	Yes				
	8	70	88,6	7,8	75,7	Yes	98,6	12,6	81,4	Yes	98,6	11,0	87,1	Yes
	9	52	69,2	5,6	55,8	Yes	88,5	3,5	53,8	Yes	100,0	3,2	50,0	Yes
	10	60	56,7	8,8	50,0	Yes	85,0	17,2	78,3	Yes	95,0	21,3	81,7	Yes
	11	57	80,7	8,4	63,2	Yes	96,5	9,8	59,6	Yes	96,5	6,6	59,6	Yes
	12	84	66,7	5,3	47,6	Yes	76,2	2,7	25,0	Yes	96,4	4,4	56,0	Yes
	13	70	70,0	4,9	48,6	Yes	81,4	2,6	25,7	Yes	98,6	3,8	45,7	Yes
	14	624	71,6	10,0	64,4	Yes	90,7	13,9	76,4	Yes	95,0	12,8	84,0	Yes
	15	70	78,6	6,3	67,1	Yes	81,4	7,5	62,9	Yes	71,4	5,7	60,0	Yes
	16	72	100,0	4,7	40,3	Yes	100,0	2,0	20,8	Yes	100,0	3,8	38,9	Yes
	17	117	88,9	4,4	47,0	Yes	73,5	3,0	30,8	Yes	88,0	1,8	16,2	Yes
	18	51	100	2,4	35,3	Yes	100,0	11,1	74,5	Yes	98,0	6,3	68,6	Yes
	19	81	95,1	13,8	70,4	Yes	91,4	22,6	67,9	Yes	71,6	8,2	46,9	Yes
	20	65	98,5	11,3	66,2	Yes	89,2	54,2	83,1	Yes	87,7	7,3	55,4	Yes
	21	61	95,1	10,2	78,7	Yes	88,5	9,8	72,1	Yes	98,4	8,5	73,8	Yes
	22	64	100,0	4,8	50,0	Yes	92,2	29,6	70,3	Yes	82,8	7,0	57,8	Yes
	23	59	98,3	8,0	67,8	Yes	98,3	24,4	83,1	Yes	98,3	13,0	84,7	Yes
	24	57	98,2	10,9	71,9	Yes	100,0	13,9	77,2	Yes	100,0	8,9	68,4	Yes
	25	60	96,7	3,1	28,3	Yes	100,0	7,0	53,3	Yes	66,7	3,2	31,7	Yes
	26	56	100,0	19,1	73,2	Yes	100,0	10,8	66,1	Yes	98,2	3,2	39,3	Yes

CHMP criterion			≥60%	≥ 2	≥30%		≥60%	≥ 2	≥30%		≥60%	≥ 2	≥30%	
	27	66	63,6	2,0	12,1	Yes	68,2	2,5	22,7	Yes	28,8	2,8	25,8	Yes
	28	56	94,6	9,3	58,9	Yes	94,6	12,3	69,6	Yes	96,4	6,3	62,5	Yes
	29	180	82,8	6,7	63,9	Yes	77,8	3,6	43,9	Yes	92,2	5,3	61,7	Yes
	30	102	80,4	10,6	69,6	Yes	77,5	2,6	31,4	Yes	100,0	3,4	49,0	Yes
	31[2]	51	62,7	3,1	35,3	Yes	86,3	2,3	21,6	Yes				
	32	78	42,3	1,9	21,8	No	60,3	1,6	16,7	Yes	65,4	2,0	19,2	Yes
	33	50	66,	4,8	50,0	Yes	76,0	2,6	24,0	Yes	100,0	2,9	32,0	Yes
	34	57	64,9	9,1	57,9	Yes	73,7	14,3	68,4	Yes	89,5	23,5	73,7	Yes
	35	66	93,9	5,2	53,0	Yes	86,4	3,7	37,9	Yes	93,9	6,4	53,0	Yes
	36	109	76,1	2,2	25,7	Yes	54,1	1,5	8,3	No	96,3	1,4	8,3	Yes
>60 years	37	100	88,	4,5	53,0	Yes	99,0	1,9	21,0	Yes	74,0	2,6	26,0	Yes
	38	55	100,0	1,4	9,1	Yes	100,0	2,0	18,2	Yes	94,5	1,9	10,9	Yes
	39	442	99,8	3,4	50,2	Yes	98,4	2,6	33,0	Yes	97,5	3,2	44,8	Yes
	40	56	98,2	8,8	28,6	Yes	96,4	12,3	17,9	Yes	96,4	6,6	35,7	Yes
	41	74	74,3	3,4	28,4	Yes	62,2	3,7	33,8	Yes	47,3	2,6	18,9	Yes
	42	105	87,6	5,4	54,3	Yes	42,9	3,2	21,0	Yes	94,3	2,9	33,3	Yes
	43	57	87,7	6,0	61,4	Yes	82,5	7,9	61,4	Yes	100,0	6,3	61,4	Yes
	44	75	93,3	7,0	57,3	Yes	57,3	3,9	37,3	Yes	92,0	5,2	50,7	Yes
	45	55	94,5	6,1	56,4	Yes	94,5	8,3	58,2	Yes	94,5	7,9	60,0	Yes
	46	53	98,1	6,1	60,4	Yes	98,1	5,9	50,9	Yes	100,0	5,1	50,9	Yes
	47	63	93,7	3,3	28,6	Yes	100,0	3,9	42,9	Yes	41,3	1,9	11,1	No
	48	58	89,7	10,8	67,2	Yes	87,9	7,0	50,0	Yes	89,7	6,3	44,8	Yes

[1], marked entry: trial result not meet the CHMP criterion.

[2], bivalent vaccine without B component.

Abbreviations: PR=protection rate, MFI=mean fold increase, RR=response rate, Reg.met = CHMP requierment met

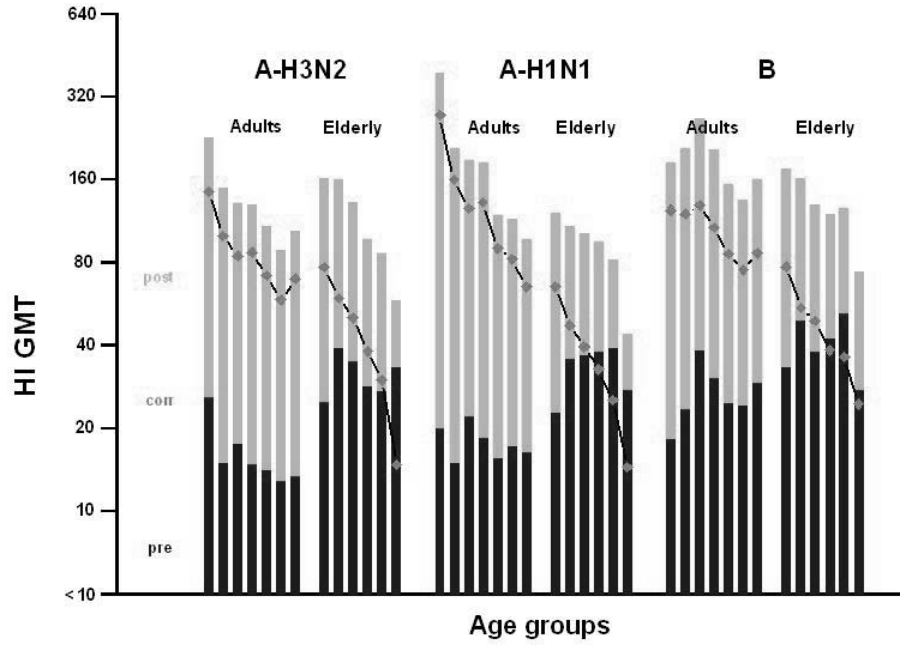
seroprotection rates were slightly lower in the elderly. In trial specific linear regression models pre-vaccination titres were a predictor of post-vaccination titres in 70% of the trials.

The influence of age on pre- and post-vaccination titres in adults and elderly

Figure 4 shows age-specific pre- and post-vaccination GMT-values, and the post-vaccination GMT-values corrected for pre-vaccination titres. The adult group was divided into 7 five-year age bands (18-23 years to 54-59 years), and the elderly age class into 6 five-year age bands (61-66 years to 91-96 years).

Pre-vaccination GMT-titres were higher in the elderly than in the adults, but did not show a clear trend. Post-vaccination GMT-titres decreased with age in adults and elderly for all three subtypes (up to 4-fold for the A/H1N1 subtype in adults). This pattern was even clearer for pre-vaccination corrected GMT-values. In terms of GMT increase, persons of ≥80 years responded poorly to vaccination.

Figure 4. Geometric mean titres within 5-year band age classes.



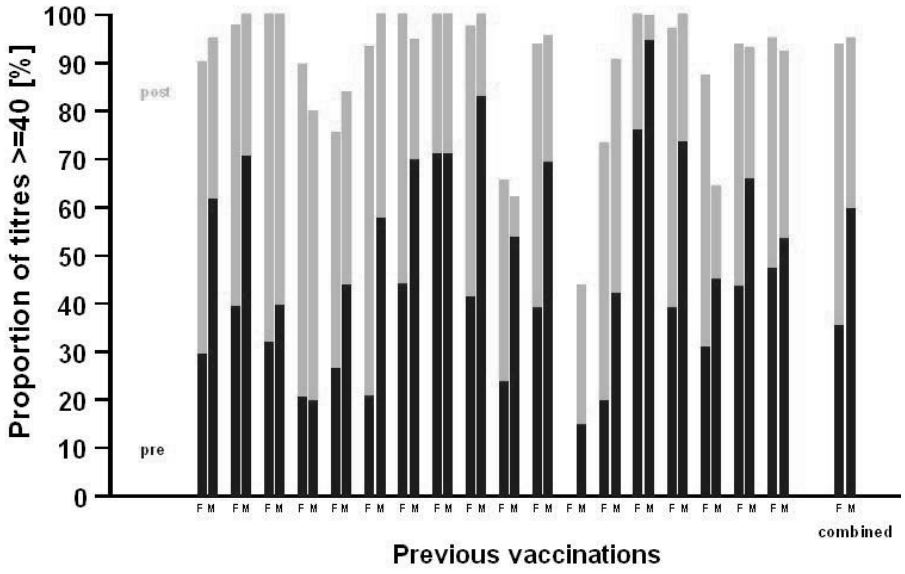
Abbreviations: HI: haemagglutinin inhibition; GMT: geometric mean titre

Pre = prevaccination geometric mean titre of the A/H3N2, a/H1N1 or B strain presented for the different age categories.

Post = postvaccination geometric mean titre of the A/H3N2, a/H1N1 or B strain presented for the different age categories.

Corr. = corrected GMT = postvaccination GMT corrected for prevaccination GMT by linear regression.

Figure 5. Pre- and post-vaccination seroprotection titres for A/H3N2 subtype of 18 individual trials for which vaccination history data were available.



Abbreviations : F: first vaccination group; M, multiple vaccinations group; pre: prevaccination; post: postvaccination.

The influence of previous vaccinations on post-vaccination titres

Vaccination history (influenza vaccination in the year(s) before the trial) was recorded in 22 trials. In 4 trials the number of subjects with previous vaccination was smaller than 5; these trials were excluded because of statistical reasons. The remaining 18 trials included 1,614 subjects. Subjects were either never previously vaccinated (single vaccination group), or vaccinated in the year prior to the trial, or previously vaccinated but not in the year prior to the trial. Since there was no statistically significant difference between the latter two groups (not shown), they were combined (multiple vaccination group). In a meta-analysis (DerSimonian & Laird, see Methods) the pre-vaccination seroprotection rate was 22.6% ($CI_{95\%}$: 17.0% - 28.2%) higher in those with multiple vaccinations as compared to those with no history of vaccination. No difference in postvaccination seroprotection rates was observed (1.1%; $CI_{95\%}$:-1.7% - 4.0%). Similar patterns were found for pre- and post-vaccination GMT (combined by the inverse variance-weighted method), and for A/H1N1 and B (not shown).

Discussion

This study indicates that for serological annual update trials of inactivated influenza vaccines the influence of population characteristics on the outcomes precludes any assessment of the immunogenic potential of the vaccine. All but 3 trials fulfilled at least one CHMP criterion for all 3 strains [3].

In an effort to adjust for confounding population factors, seroresponse was introduced in the CHMP NfG in addition to seroprotection rate. Furthermore, studies were stratified into 2 age classes and inclusion of only healthy individuals should preclude an impact of underlying disease on the immune responsiveness. In our study, we strictly applied the CHMP criteria, which may not have been the case for the original studies. For the purpose of annual relicensure it is thus possible that different conclusions were drawn.

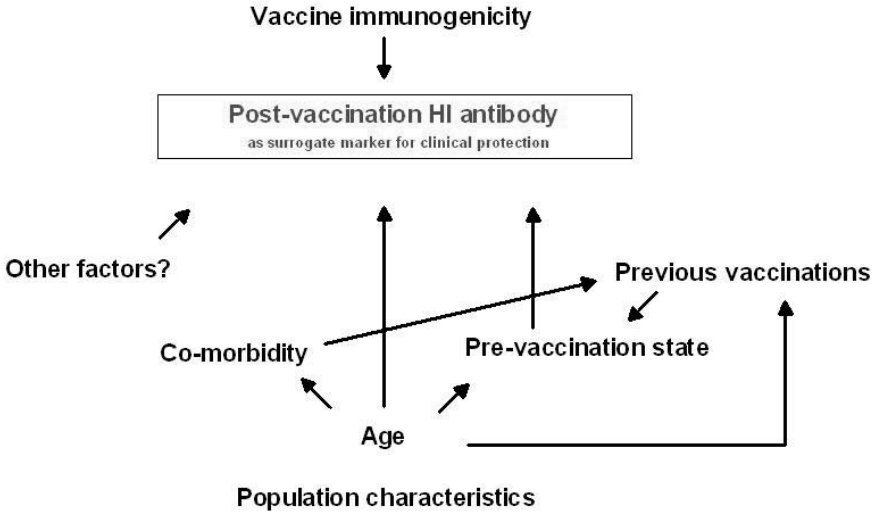
It is doubtful whether these measures were sufficient to control for confounding factors. First, in persons with high pre-vaccination titres only a small amount of newly induced antibody is enough to reach the required post-vaccination antibody titres. This precludes a reliable assessment of the antibody-inducing potency of a vaccine. Although the quotient of post- and pre-vaccination titre should correct for the pre-vaccination titre, it has been shown to be insufficient since the mean fold increase and response rate remain dependent on pre-vaccination titre [4]. Stratification in only two age groups was insufficient to control for the age dependency of the antibody response.

Furthermore, the CHMP inclusion criterion of being 'healthy' is vague and may not exclude unapparent pathological conditions. Our data were not detailed enough to assess this possibility. Although we adjusted for health status by excluding every individual with recorded comorbidity, we cannot exclude residual comorbidity. Comorbidity of individual subjects was recorded in a minority of the trials, and in none of the trials conducted prior to 1995.

The CHMP NfG does not consider other possibly relevant population characteristics such as previous vaccinations and genetic variation between individuals. We found no clear influence of vaccination history on post-vaccination titres for the combined trials. This is in accordance with earlier observations [16]. However, for a single trial it does not exclude unwanted large variation by its strong positive influence on the pre-vaccination state.

It should be emphasised that all the summarised population characteristics are correlated. For example, pre-vaccination titre, co-morbidity and previous vaccinations may themselves be dependent on age, and again pre-vaccination titres on previous vaccinations, resulting in a complex pattern of interactions (figure 6). Interpretation is further complicated by the fact that correlations between vaccine strains and

Figure 6. Possible population characteristics that may influence postvaccination immune response to influenza vaccination



circulating strains might play a more profound role in clinical performance of influenza vaccines than previously assumed, especially in those annually revaccinated [17, 18].

Immunogenicity of influenza vaccines is determined each year in open label uncontrolled trials of limited size. Due to lack of a comparator group and lack of random treatment assignment the immunogenic properties of the vaccines cannot be assessed unbiased. Stratification and restrictions are measures to reduce confounding but within strata residual confounding may occur.

In a time which is characterized by a need for evidence-based medicine, and in view of the serious limitations of these trials, the final question is "what do we expect from an annual update trial?"

Each influenza vaccine qualifying for annual relicensure has to proof its quality, immunogenicity and safety in a sufficiently large dataset. The subsequent annual update trials are no more than a model to address consistency of immunogenicity. The selected population in such a study is of major importance, as it has to be the most optimal for the desired outcome, through minimal interference of disturbing population characteristics. For influenza vaccines this means a relatively naïve healthy individual, e.g., a never influenza vaccinated healthy young adult. In this perspective, the observed reduction of the median age of the study populations in the more recent years could be interpreted as an improvement to address the objectives.

It is noted that the EU is the only region in which annual update trials are presently requested. Other regulatory authorities only require assessment of the potency of

the annually produced influenza vaccines by in vitro tests using a standardised SRH assay [3], which is also required in the CHMP NfG and, which in several studies is validated for protection [19-21].

In conclusion, the need and design of annual update trials of inactivated influenza vaccines may be questioned and should be rediscussed. The present practice of follow-up of the CHMP NfG criteria inhibits progress for inactivated influenza vaccines, as it does not encourage companies and investigators to explore other possibly relevant immunological parameters to support new applications or specific clinical claims [22, 23].

References

1. van Essen, G.A., et al., *Influenza vaccination in 2000: recommendations and vaccine use in 50 developed and rapidly developing countries*. Vaccine, 2003. 21(16): p. 1780-5.
2. Govaert, T.M., et al., *The efficacy of influenza vaccination in elderly individuals. A randomized double-blind placebo-controlled trial*. JAMA, 1994. 272(21): p. 1661-5.
3. *Committee for Proprietary Medicinal Products (CHMP). Note for Guidance on Harmonisation of Requirements for Influenza Vaccines*. (CHMP/BWP/214/96), (adopted March 1997).
4. Beyer, W.E., et al., *Seroprotection rate, mean fold increase, seroconversion rate: which parameter adequately expresses seroresponse to influenza vaccination?* Virus Res, 2004. 103(1-2): p. 125-32.
5. McElhaney, J.E., et al., *The effect of influenza vaccination on IL2 production in healthy elderly: implications for current vaccination practices*. J Gerontol, 1992. 47(1): p. M3-8.
6. McElhaney, J.E., et al., *Vaccine-related determinants of the interleukin-2 response to influenza vaccination in healthy young and elderly adults*. Vaccine, 1995. 13(1): p. 6-10.
7. Powers, D.C. and R.B. Belshe, *Effect of age on cytotoxic T lymphocyte memory as well as serum and local antibody responses elicited by inactivated influenza virus vaccine*. J Infect Dis, 1993. 167(3): p. 584-92.
8. Provinciali, M., et al., *Impaired antibody response to influenza vaccine in institutionalized elderly*. Ann N Y Acad Sci, 1994. 717: p. 307-14.
9. Remarque, E.J., et al., *Correlation between the antibody response to influenza vaccine and helper T cell subsets in healthy aging*. Vaccine, 1996. 14(2): p. 127-30.
10. Deng, Y., et al., *Age-related impaired type 1 T cell responses to influenza: reduced activation ex vivo, decreased expansion in CTL culture in vitro, and blunted response to influenza vaccination in vivo in the elderly*. J Immunol, 2004. 172(6): p. 3437-46.
11. Kohut, M.L., et al., *Exercise and psychosocial factors modulate immunity to influenza vaccine in elderly individuals*. J Gerontol A Biol Sci Med Sci, 2002. 57(9): p. M557-62.
12. Kubiet, M.A., et al., *Serum antibody response to influenza vaccine in pulmonary patients receiving corticosteroids*. Chest, 1996. 110(2): p. 367-70.
13. Carson, P., et al., *Immune function and vaccine responses in healthy advanced elderly persons*. Arch.Intern.Med, 2000(160): p. 2017-24.
14. Sutton, A., et al., *Methods for Meta-Analysis in Medical Research* . Chichester:John Wiley & Sons, Ltd., 2000: p. page 58.
15. DerSimonian, R. and N. Laird, *Meta-analysis in clinical trials*. Contr.Clin.Trials, 1986(7): p. 177-88.
16. Beyer, W.E., et al., *Effects of repeated annual influenza vaccination on vaccine sero- response in young and elderly adults*. Vaccine, 1996. 14(14): p. 1331-9.
17. Smith, D.J., et al., *Variable efficacy of repeated annual influenza vaccination*. Proc Natl Acad Sci U S A, 1999. 96(24): p. 14001-6.
18. Smith, D.J., et al., *Mapping of the antigenic and genetic evolution of influenza virus*. Science, 2004. 305: p. 371-376.
19. Delem, A. and D. Jovanovic, *Correlation between rate of infection and preexisting titer of serum antibody as determined by single radial hemolysis during and epidemic of influenza A/Victoria/3/75*. J Infect Dis, 1978. 137(2): p. 194-6.
20. Goodeve, A., et al., *A graded-dose study of inactivated, surface antigen influenza B vaccine in volunteers: reactogenicity, antibody response and protection to challenge virus infection*. J Hyg (Lond), 1983. 90(1): p. 107-15.
21. Al-Khayatt, R., R. Jennings, and C.W. Potter, *Interpretation of responses and protective levels of antibody against attenuated influenza A viruses using single radial haemolysis*. J Hyg (Lond), 1984. 93(2): p. 301-12.

22. De Bruijn, I.A., et al., *Virosomal influenza vaccine: a safe and effective influenza vaccine with high efficacy in elderly and subjects with low pre-vaccination antibody titers.* Virus Res, 2004. 103(1-2): p. 139-45.
23. Frey, S., et al., *Comparison of the safety, tolerability, and immunogenicity of a MF59-adjuvanted influenza vaccine and a non-adjuvanted influenza vaccine in non-elderly adults.* Vaccine, 2003. 21(27-30): p. 4234-7.

7

Do outcomes of serological studies with influenza vaccines in elderly predict clinical protection after annual vaccination? Review of the literature

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IM Uhnoo
J van der Noordaa
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BHCh Stricker

Abstract

Context

Efficacy of strain variations in influenza vaccines is measured by serological parameters. Little is known about their validity as correlate for clinical protection in elderly individuals, especially when administered annually.

Objective

The objective of this review was to investigate the impact of annual vaccination on serological and clinical parameters, and the relevance of using serological outcomes as proxy for clinical protection.

Methods

Publications of studies based upon use of inactivated trivalent influenza vaccines in elderly subjects and relevant reviews were selected from a MEDLINE search until 2005. For this purpose, publications needed to describe total number of subjects and those with the outcome of interest for the exposed and non-exposed group.

Results

In 23 serology publications no correlations were found between postvaccination seroprotection rate and seroresponse. The response rates in uncontrolled trials appeared less heterogeneous than in controlled trials. Thirty publications addressing clinical outcomes showed a significant protective effect with regard to death, pneumonia and influenza. In the presence of a vaccination history, serological responses did not adequately predict clinical response. There were only a few studies which addressed both serological and clinical parameters. None of these confirmed the validity of the serological parameter as a correlate for clinical protection.

Conclusions

This review supports the clinical benefit of annual influenza vaccination in elderly, but does not support the use of serological data as a proxy for clinical outcomes.

Introduction

Annual influenza epidemics are associated with substantial morbidity and mortality, especially in elderly and in those with underlying diseases [1-4]. Among older community dwelling and institutionalised individuals, respiratory virus infections are common. A substantial proportion of these are caused by influenza and associated with respiratory complications, hospitalisations and excess deaths [5-13].

Influenza vaccination programs focus on elderly persons and on those with risk factors. Intramuscularly administered inactivated influenza vaccines primarily stimulate production of immunoglobulin G (IgG) antibodies. Age-related decline in antibody response, or immunosenescence, has been reported in several publications [14-20], but not in all [21-24]. Contrary to the humoral immunity which is strain-specific, T cell mediated immunity is cross-reactive with many other strains of influenza A and B. This is supposed to offer protection against many influenza virus strains. Age, lifestyle and health status may affect T cell function and thus humoral responses to influenza viral antigens [21, 25-36], although preserved immune function is reported in those reaching advanced age in overall good health [37].

Strategies to improve age-related decreases in vaccine efficacy include annual revaccination [20, 38, 39], increased antigenic content of the vaccines [40-43], use of whole cell vaccines [44], use of booster doses [43, 45-49], intranasal administration [50], use of adjuvants [51, 52] and other immunomodulators [53, 54], live attenuation of the virus [46, 55, 56], and alternative routes of administration [57]. However, up till now, only annual revaccination appears to be an effective strategy, although this is not generally accepted [53, 58].

Trials performed for annual relicensure of the inactivated influenza vaccines are based upon predefined serological criteria described in the so-called "CHMP (Committee of Human Medicinal Products) Note for Guidance on harmonisation of requirements for influenza vaccines" [59]. Most trials seem to meet these criteria [20, 39, 51, 60]. However, their clinical relevance is not always obvious [23, 61, 62]. Serological data are only incidentally supplemented by data on clinical protection [51, 55, 56, 63-68]. A meta-analysis of single versus multiple vaccination indicated no specific benefit of revaccination according to serological parameters [23]. But despite their limitations, CHMP criteria are often used in experimental trials as a proxy for clinical protection.

Early observational studies in institutionalised and community dwelling elderly supported the benefit of annual mass influenza vaccination [63, 69-73]. A large number of observational studies report a variety in protective effects of influenza vaccination on clinically relevant endpoints, such as influenza like illness, (hospitalisations for) pneumonia, cardiovascular diseases and mortality [55, 56, 68, 70, 73-96]. Meta-analy-

ses that included a limited number of studies showed an overall benefit on mortality and hospitalisations for pneumonia and other respiratory infections [97-100].

The serological responses in trials are generally used as a correlate for clinical protection [33, 56]. However, they are not unambiguous [19, 101]. Out of a number of studies and one meta-analysis addressing both clinical outcomes and serological responses, [23, 33, 56, 63-65, 76, 102] only one study reported a positive correlation [56]. Antigenic variations of vaccine strains and circulating influenza strains and the individuals' vaccination and infection history are confounding factors in interpreting the validity of serological data as a correlate for clinical protection [53, 74, 75, 103-105].

The objective of this review is to investigate the impact of annual vaccination on serological and clinical parameters and the validity of using serological endpoints as a surrogate parameter.

Selection of publications

A Medline search was performed using one or more of the following terms in association with "influenza": "vaccine", "vaccination", "clinical", "Influenza Like Illness", "pneumonia", "hospitalisation", "mortality", "death", "serology", "serological", "elderly" and "correlation". Publications of studies on use of inactivated trivalent influenza vaccines in elderly subjects as well as relevant reviews were selected. Their reference lists were further investigated for additional publications.

Relevant publications with quantitative data are listed in table 1 when it concerned serological studies and tables 2-4 when the publications had clinical outcomes. To be considered for inclusion in these tables, a publication needed to describe absolute numbers of vaccinated and non-vaccinated subjects and the number with the outcome of interest. For serological studies this was postvaccination response rate, defined as a ≥4 fold increase in geometric mean titre (GMT) and postvaccination seroprotection rate, defined as postvaccination GMT ≥40 by Haemagglutination Inhibition (HI) assay. Clinical endpoints were death, pneumonia or confirmed influenza associated respiratory infections.

Seroprotection and seroresponse

The CHMP criteria require a post vaccination seroprotection rate of 60% or more in elderly aged 61 years or older [59]. However, this is not always achieved [20, 25, 26, 38, 41, 52, 55, 56, 107] . A randomised placebo controlled trial in elderly general

practioner's patients showed relatively low seroprotection rates following vaccination, ranging from 43-68%. In patients not previously vaccinated, seroprotection rates varied between 46-69% whereas in those with a vaccination history this was only 19-52% [64] (table 1). Others found seroprotection rates well above the predefined CHMP limit of 60% [19, 45, 47, 49-51, 53, 60, 68, 96, 108].

Efforts to improve the seroprotection rate were generally not successful. In a review summarising dose response studies only 3/26 study groups showed a significant positive dose response relationship [42]. Also other strategies, such as adjuvants [51], booster doses [45, 47, 49, 68], live attenuation of the virus [56] did not lead to significant increases in seroprotection rates. However, the use of higher vaccine dosages and adjuvants was again proposed as a possibility to improve vaccine-induced immune responses in the elderly [109].

According to the CHMP criteria, seroresponse rate should exceed 30% in elderly. In several studies the response rate is inversely related to the prevaccination antibody titre. Individuals with low prevaccination titres tend to have a higher postvaccination titre, and thus a higher response rate [46]. Also response rate has shown to be highly variable between studies (see table 1). Non-response to all 3 strains has been reported to occur in up to 46% of the vaccinees [16].

In the 23 publications summarised in table 1, we identified 42 different mutually exclusive treatment periods and/or subgroups. Logistic regression analyses performed on the outcomes indicated that there was no correlation between postvaccination seroprotection rate and response rate for the A/H3N2, A/H1N1 and B strain.

Clinical protection

Several studies addressed confirmed influenza infections during an influenza epidemic period in community dwelling elderly (table 2). In a randomised controlled trial, influenza vaccination resulted in a 50% reduction of serologically confirmed influenza infections ($CI_{95\%}$: 39-65%). In 3 other studies, influenza vaccination was as effective in reducing confirmed influenza infections [87, 91, 110]. The use of live attenuated vaccine with or without simultaneous inactivated influenza vaccine administration resulted in similar incidences of serologically confirmed influenza infections in elderly COPD patients [56]. As clinical detection of influenza like illness alone often fails to show a significant benefit from vaccination [9, 65, 69, 90, 91, 102, 112], the use of serological or virological methods to ascertain influenza infection is advised to increase diagnostic sensitivity [110, 113, 114].

Table 1: Overview and descriptive results of serological trials

Ref.	Author	Infuenza season(s)	In/Out patients	Health status	Design	Follow-up (wks)	No.	POSTVACCINATION RESPONSE					
								Seroprotection (%)			Seroresponse (%)		
								H3	H1	B	H3	H1	B
First vaccination													
[64]	Govaert	1991-'92	Out	Mixed	Controlled	3	788	69	46	50	-	-	-
[53]	Ben-Yehuda	1995-'96	Out	Mixed	Controlled	4	9	89	100	22	-	-	-
[20]	De Bruyn	1990-'93	Out	Mixed	Uncontrolled	3	138	70	23	33	-	-	-
Revaccination													
[32]	Powers	1990-'91	Out	Healthy	Controlled	12	17	-	-	-	29	29	59
[42]	Palache	1988-'99	In	Comorbid	Controlled	3	70	76	33	67	-	-	-
[50]	Muszkat	1998-'99	In	Comorbid	Controlled	3	22	77	68	55	55	73	18
[107]	Lorio	1995-'96	In	Comorbid	Controlled	4	80	70	76	84	64	58	69
[40]	Remarque	1988-'99	In	Comorbid	Uncontrolled	3	28	-	-	-	21	-	-
[64]	Govaert	1991-'92	Out	Mixed	Controlled	3	118	52	20	19	-	-	-
[45]	Gross	1985-'86	Out	Mixed	Controlled	4	54	81	94	87	-	-	-
[53]	Ben-Yehuda	1995-'96	Out	Mixed	Controlled	4	26	50	88	65	-	-	-
[107]	Lorio	1995-'96	Out	Mixed	Controlled	4	51	41	63	57	51	24	51
[20]	De Bruyn	1990-'93	Out	Mixed	Uncontrolled	3	138	81	28	78	-	-	-
[38]	Gardner	1994-'95	Out	Mixed	Uncontrolled	6	92	43	84	58	28	10	12
[38]	Gardner	1995-'96	Out	Mixed	Uncontrolled	6	92	49	82	82	29	10	29
[38]	Gardner	1996-'97	Out	Mixed	Uncontrolled	6	92	76	76	68	32	12	12

No differentiation in vaccination status													
[51]	Squarcione	1998-99	Out	Healthy	Controlled	3	591	90	72	29	60	64	15
[46]	Powers	1988-'89	Out	Healthy	Controlled	4	26	-	-	-	23	35	-
[47]	Buxton	1998-'99	Out	Healthy	Controlled	24	27	93	81	56	-	-	-
[52]	Frech	2002-'03	Out	Healthy	Uncontrolled	3	55	89	58	29	36	40	38
[108]	Rastogi	1992-'93	Out	Healthy	Uncontrolled	4	95	78	63	89	-	-	-
[55]	Gorse	1994-'95	Out	Comorbid	Controlled	1	13	-	-	-	23	23	-
[19]	Glathe	1991-'92	In	Comorbid	Controlled	4	58	100	100	97	62	78	83
[68]	Wongsurakiat	1997-'98	Out	Comorbid	Controlled	4	62	84	74	44	74	77	48
[55]	Gorse	1994-'95	Out	Comorbid	Controlled	4	13	-	-	-	62	46	-
[56]	Gorse	1998-'99	Out	Comorbid	Controlled	4	32	-	-	-	53	59	34
[49]	Vogtländer	1998-'99	In	Comorbid	Controlled	8	44	77	45	86	25	43	23
[108]	Rastogi	1992-'93	In	Comorbid	Uncontrolled	4	23	74	87	83	-	-	-
[19]	Glathe	1991-'92	Out	Mixed	Controlled	4	70	87	79	91	79	40	47
[26]	Brydak	1999-'00	In	Mixed	Controlled	4	45	91	42	42	91	42	42
[25]	Zei	1989-'90	Out	Mixed	Controlled	5	60	52	47	3	17	40	5
[25]	Zei	1989-'90	Out	Mixed	Controlled	5	24	71	71	42	38	54	50
[26]	Brydak	1999-'00	In	Mixed	Controlled	24	45	84	16	18	82	16	18
[16]	Goronzy	<1991	Out	Mixed	Uncontrolled	4	153	-	-	-	42	26	33
[76]	Odelin	1987-'95	In	Mixed	Uncontrolled	4	477	-	-	-	49	31	31
[38]	Gardner	1993-'94	Out	Mixed	Uncontrolled	6	92	57	45	48	39	32	10
							Median	77.9	70.8	57.6	38.3	40.0	30.1
							IQR	17.2	36.1	41.0	34.4	34.0	34.5
							Min	41.2	15.6	3.33	0	1.6	0
							Max	96.3	100	93.2	91.1	7.6	82.8

Abbreviations: IQR – interquartile range = Q75 – Q25, measure of variation
Patients: In = hospitalised or institutionalised;
Out = GP practice of outpatient clinic
Controlled design = any control group, i.e. placebo, other active group, other dose, booster vs. non-booster dose
Seroprotection: HI-titre≥40
Seroresponse: ≥ 4-fold increase in GMT when prevaccination GMT ≥10, or seroconversion (GMT<10 before vaccination to ≥ 40 postvaccination

Table 2: Overview and descriptive confirmed influenza infection results from selected clinical studies

Influenza											
		season		health	mean age		vaccine		control		
Ref.	Author		Design	status	vaccine	control	yes	no	yes	no	Crude OR (± $CI_{95\%}$)
GP practice or outpatient setting											
[65]	Govaert	'91-'92	RCT	Mixed	67.3	67	41	886	80	831	0.50 (0.35-0.73)
[87]	Nicholson	'93-'94	Cohort	Mixed	>65		1	440	19	420	0.05 (0.01-0.39)
[91]	Carrat	'95-'96	CC	Mixed			13	155	33	133	0.39 (0.21-0.71)
Hospitalised or institutionalised patients											
[61]	Nicholson	'88-'89	CC	Comor	85.3	85.3	1	72	5	31	0.10 (0.01-0.81)
[74]	Dindenaud	'89-'90	Cohort	Mixed	66.8		12	69	2	32	2.52 (0.60-10.7)
[86]	Christenson	'98-'99	Cohort	Mixed	75.2	75.8	36	23188	386	159000	0.64 (0.46-0.90)
[111]	Deguchi	'98-'99	Cohort	Mixed	82		256	10483	694	11029	0.40 (0.35-0.46)
Abbreviations: REF.: reference; GP: general practitioner; RCT: randomised controlled trial; CC: case control; OR: odds ratio							Influenza $_{fixed}$				0.42 (0.37-0.48)
							Influenza $_{random}$				0.45 (0.31-0.65)
Fixed model / random model according to DerSimonian and Laird[106]							heterogeneity				p=0.003

Most data in elderly, however, concern the effect of influenza vaccines on mortality or influenza associated respiratory complications (see tables 3 & 4). Some studies focus on specific patient populations [55, 56, 68, 94-96].

Large cohort studies have been performed using computerized datasets from managed care organizations [11, 70, 73, 77-84] , insurance companies [85], primary care settings [87-93, 115], and through media campaigns [86]. The sample sizes achieved with these studies are reflected in the higher precision of the outcomes (see tables 3 & 4). Several large cohort studies report a protective efficacy (i.e., 1-relative risk) of influenza vaccination against (hospitalisations for) pneumonia with or without influenza in elderly persons, which is generally lower in persons with predefined comorbidity compared to the "healthy" elderly [77-79, 82, 85, 88, 90, 116] . But a stronger benefit for elderly with comorbidity has been reported as well [73, 81]. The studies included in table 3 showed an overall protective benefit of influenza vaccination against pneumonia, which appeared to be stronger in healthy elderly (OR, 0.35, $CI_{95\%}$: 0.32-0.39) than in those with comorbidity (OR, 0.74; $CI_{95\%}$: 0.70-0.78). In a population receiving both influenza vaccine and polysaccharide pneumococcal vaccine, the unadjusted reduction in hospitalisation was 29% ($CI_{95\%}$: 24-34%) for pneumonia and 46% ($CI_{95\%}$: 34-56%) for influenza with or without pneumonia [86]. In institutionalised elderly, whose health status is less favourable, the benefit of influenza vaccination has been positively associated with vaccination coverage, revaccination status, and/or antigenic match between circulating strain and vaccine strain [69, 71, 74, 117, 118]. A booster dose had no significant clinical benefit over a single dose [111].

Table 3: Overview and descriptive pneumonia results from selected clinical studies

pneumonia											
				health	mean age		vaccine		control		
Ref.	Author	season	Design	status	vaccine	control	yes	no	yes	no	Crude OR (± $CI_{95\%}$)
Data from GP practice or outpatient setting											
[90]	Voordouw	'96-'97	Cohort	Healthy	74.7	74.7	28	5321	54	5996	0.59 (0.37-0.92)
[90]	Voordouw	'96-'97	Cohort	Comor.	74.7	74.7	44	3518	29	2832	1.22 (0.76-1.94)
[9]	Connolly	'89-'90	CC	Mixed			8	334	7	335	1.14 (0.42-3.12)
[112]	Allsup	'99-'00	RCT	Mixed	68.9	69.1	0	552	0	177	-
Hospital data or institutionalised patients											
[73]	Barker	'68-'69	Cohort	Healthy	71.3	71.2	3	957	13	4847	1.17 (0.33-4.09)
[73]	Barker	'72-'73	Cohort	Healthy	72.4	71.5	0	740	13	5187	-
[82]	Mullooly	'80-'89	CC	Healthy	> 65	> 65	128	31620	2291	116674	0.21 (0.18-0.25)
[94]	Nichol	'93-'96	Cohort	Healthy	73.5	532	56	1310	29	503	0.75 (0.49-1.16)
[81]	Hak	'96-'97	Cohort	Healthy	74.2	74	201	37492	254	30589	0.65 (0.54-0.78)
[81]	Hak	'97-'98	Cohort	Healthy	74.3	73.9	164	33991	267	32222	0.58 (0.48-0.71)
[73]	Barker	'68-'69	Cohort	Comor.	72.2	73	4	1116	15	2805	0.67 (0.22-2.02)
[73]	Barker	'72-'73	Cohort	Comor.	74.4	74.1	2	1098	24	3676	0.28 (0.07-1.18)
[82]	Mullooly	'80-'89	CC	Comor.	> 65	> 65	734	36783	1474	61330	0.83 (0.76-0.91)
[81]	Hak	'96-'97	Cohort	Comor.	74.2	74	695	32617	811	20315	0.54 (0.49-0.60)
[81]	Hak	'97-'98	Cohort	Comor.	74.3	73.9	1129	56717	995	32969	0.67 (0.61-0.73)
[63]	Howells	'71-'72	Cohort	Mixed	80.1	76.9	2	132	18	338	0.30 (0.07-1.26)
[63]	Howells	'72-'73	Cohort	Mixed	78.7	79.8	3	120	28	239	0.23 (0.07-0.75)
[63]	Howells	'73-'74	Cohort	Mixed	78.7	78.8	0	183	11	276	-
[71]	Saah	'79-'80	Cohort	Mixed	84	83	11	208	20	214	0.59 (0.29-1.20)
[71]	Saah	'80-'81	Cohort	Mixed	83	84	12	232	11	203	0.96 (0.43-2.12)
[71]	Saah	'81-'82	Cohort	Mixed	83	85	9	216	16	210	0.57 (0.26-1.25)
[69]	Patriarca	'82-'83	Cohort	Mixed	82.3	80.4	22	526	45	425	0.42 (0.26-0.69)
[84]	Fedson	'82-'83	CC	Mixed	69.8	68.2	283	2336	754	7098	1.13 (0.99-1.28)
[84]	Fedson	'85-'86	CC	Mixed	71.1	71.1	370	2047	1008	6241	1.10 (0.99-1.23)
[70]	Foster	'89-'90	CC	Mixed	78.5		135	188	524	718	0.99 (0.86-1.14)
[93]	Mangtani	'90-'99	Cohort	Mixed	> 65		1993	143713	3177	270865	1.18 (1.12-1.25)
[78]	Nichol	'90-'93	Cohort	Mixed	72	72.5	166	41252	297	36812	0.50 (0.41-0.61)
[77]	Nichol	'90-'91	Cohort	Mixed	72	72.1	29	11454	81	13968	0.44 (0.29-0.67)
[77]	Nichol	'91-'92	Cohort	Mixed	72	72.5	83	15205	124	10957	0.49 (0.37-0.64)
[77]	Nichol	'92-'93	Cohort	Mixed	72.4	72.5	63	14584	99	11880	0.52 (0.38-0.71)
[116]	Puig	'94-'95	CC	Mixed	76.3	76.4	47	36	102	64	0.92 (0.74-1.15)
[68]	Wonsurakiat	'97-'98	RCT	Mixed	67.6	69.1	2	60	12	51	0.17 (0.04-0.73)
[86]	Christenson	'98-'99	Cohort	Mixed	75.2	75.8	335	22889	2468	156918	0.93 (0.83-1.04
[111]	Deguchi	'98-'99	Cohort	Mixed	82		32	10707	150	11573	0.23 (0.16-0.34)
[79]	Nichol	'98-'99	Cohort	Mixed	74.2	73.7	495	77243	581	61736	0.68 (0.61-0.77)
[79]	Nichol	'98-'99	Cohort	Mixed	74.2	73.4	589	86768	501	58470	0.79 (0.71-0.89)
Abbreviations: REF.: reference; GP: general practitioner; RCT: randomised controlled trial; CC: case control; OR: odds ratio							Pneumonia $_{\text{fixed model}}$				0.80 (0.78-0.83)
							Pneumonia $_{\text{random model}}$				0.63 (0.53-0.74)
Fixed model / random model according to DerSimonian and Laird[106]							heterogeneity				p<0.0001

Table 4: Overview and descriptive of mortality results from selected clinical studies

mortality											
				health	mean age		vaccine		control		
Ref.	Author	season	Design	status	vaccine	control	yes	no	yes	no	crude OR(± $CI_{95\%}$)
Data from GP practice or outpatient setting											
[90]	Voordouw	'96-'97	Cohort	Healthy	74.7	74.7	68	5281	88	5962	0.87 (0.64-1.20)
[90]	Voordouw	'96-'97	Cohort	Comorb.	74.7	74.7	75	3487	76	2785	0.79 (0.58-1.09)
[89]	Voordouw	'96-'02	Cohort	Mixed	73.1		2225	60251	1260	25252	0.75 (0.70-0.80)
[92]	Fleming	'89-'90	Cohort	Mixed	71.3	69.1	3	596	84	7848	0.47 (0.15-1.49)
[119]	Ahmed	'89-'90	CC	Mixed	82.4	83.1	57	258	178	599	0.74 (0.53-1.04)
[65]	Govaert	'91-'92	RCT	Mixed	67.3	67	6	921	3	908	1.97 (0.49-7.91)
[112]	Allsup	'99-'00	RCT	Mixed	68.9	69.1	3	549	1	176	0.96 (0.10-9.31)
[129]	Landi	'99-'00	Cohort	Mixed	78.8	80.1	167	917	192	806	0.77 (0.61-0.96)
Hospital data or institutionalised patients											
[73]	Barker	'68-'69	Cohort	Healthy	72.2	73	0	960	1	4859	-
[73]	Barker	'72-'73	Cohort	Healthy	74.4	74.1	0	740	3	5197	-
[82]	Mullooly	'80-'89	CC	Healthy	> 65	> 65	7	31741	2292	116673	0.01 (0.01-0.02)
[73]	Barker	'68-'69	Cohort	Comorb.	71.3	71.2	3	1117	8	2812	0.94 (0.25-3.57)
[73]	Barker	'72-'73	Cohort	Comorb.	72.4	71.5	0	1100	13	3687	-
[82]	Mullooly	'80-'89	CC	Comorb.	> 65	> 65	106	37411	1476	61328	0.12 (0.10-0.14)
[63]	Howells	'71-'72	Cohort	Mixed	80.1	76.9	1	133	15	341	0.17 (0.02-1.31)
[63]	Howells	'72-'73	Cohort	Mixed	78.7	79.8	3	120	22	245	0.28 (0.08-0.95)
[63]	Howells	'73-'74	Cohort	Mixed	78.7	78.8	0	183	11	276	-
[71]	Saah	'79-'80	Cohort	Mixed	84	83	2	217	12	222	0.17 (0.04-0.77)
[71]	Saah	'80-'81	Cohort	Mixed	83	84	3	241	8	206	0.32 (0.08-1.22)
[71]	Saah	'81-'82	Cohort	Mixed	83	85	3	222	5	221	0.60 (0.14 -2.53)
[69]	Patriarca	'82-'83	Cohort	Mixed	82.3	80.4	6	542	21	449	0.24 (0.10-0.59)
[33]	Provinciali	'89-'90	RCT	Mixed	81.3	81.3	29	244	3	35	1.39 (0.40-4.79)
[111]	Deguchi	'98-'99	Cohort	Mixed	82		1	10738	5	11718	0.22 (0.03-1.87)
[79]	Nichol	'98-'99	Cohort	Mixed	74.2	73.7	943	76795	1361	60956	0.55 (0.51-0.60)
[79]	Nichol	'99-'00	Cohort	Mixed	74.2	73.4	1019	86338	1026	57945	0.67 (0.61-0.73)
Abbreviations: REF.: reference; GP: general practitioner; RCT: randomised controlled trial; CC: case control; OR: odds ratio							Mortality $_{fixed\ model}$				0.58 (0.56-0.61)
Fixed model / random model according to DerSimonian and Laird[106]							Mortality $_{random\ model}$				0.42 (0.29-0.60)
							heterogeneity				p<0.0001

Overall, the studies summarised in table 4 underline the protective efficacy of influenza vaccination on mortality (OR, 0.42; $CI_{95\%}$: 0.29-0.60) (method by derSimonian and Laird) [106]. In a cohort study covering 1996-2000, (unadjusted) influenza attributable deaths were prevented in 83% [88]. On some occasions, a protective effect was seen in one season but not in the other [63, 71, 84] , in the subgroup with comorbidity but not in the subgroup without [90] and after revaccination but not after first vaccination [89]. In 2 studies the protective benefit of vaccination did not sustain when vaccination was interrupted for a year [89, 92].

Several large scale cohort studies also addressed other outcomes, such as hospitalisations for acute or chronic respiratory conditions, congestive heart failure, cerebral disease, diabetes mellitus and health care utilisation [78-80, 85, 94, 95].

The influence of vaccination history on serological response and clinical protection

Vaccination history is often recorded, but is not necessarily used as a factor in the determination of clinical effectiveness [63, 65, 68-71, 73, 79, 89-93, 119]. Although most serological studies summarised in this review addressed the vaccination status, only 4 studies distinguished between first vaccination and revaccination [19, 20, 53, 64]. In 3 studies, seroprotection rate following revaccination was lower than after first vaccination [20, 53, 64]. Based on this finding, one of the authors concluded that repeated vaccination should be avoided [53]. Similar conclusions were drawn in an early study upon impaired clinical protection of boys in a boarding school [58]. In nursing home residents, vaccination history did not result in impaired seroprotection [19]. In general, vaccination history has not shown to have a major impact on postvaccination seroprotection rate. In a cohort study covering 3 epidemic seasons, seroprotection rates tended to decrease from the individuals' first vaccination to subsequent revaccinations [39]. In another cohort study, results were more variable [20]. It was concluded that vaccination history should always be addressed as an independent factor in serological studies [39].

Another serological parameter, seroresponse rate, seems to be inversely correlated to the prevaccination antibody titre [46, 62], and higher prevaccination titres are almost exclusively observed in previously vaccinated individuals. But lower seroresponse (and seroprotection) rate is not always observed in elderly with high prevaccination antibodies. Especially in institutionalised populations, unexpected high seroresponse has been reported [19, 102, 107]. In one of these studies, also unexpectedly high clinical responses were seen [102].

In the randomised controlled trial in elderly GP practice patients, revaccination further decreased serologically confirmed influenza by 50% in first time vaccinees to a decrease by 89% in those with a vaccination history ($CI_{95\%}$: 17-99%) [65]. In adults, annual influenza vaccination did not further decrease the risk of clinically diagnosed influenza like illness [120, 121]. In a case control study revaccination reduced mortality significantly, whereas a first vaccination did not [119]. In an earlier cohort study, influenza vaccine administered directly prior to the epidemic was highly effective in reducing mortality risk in GP patients, including those with comorbidity [92, 95]. However, persons vaccinated in the year before but not directly prior to the epidemic season under study had no benefit from their previous vaccination [92]. The importance of compliance to the annual vaccination to sustain mortality risk reduction was recently reported in a cohort study covering 6 epidemic years [89].

The predictive value of serological response for clinical protection

The relationship between serological and clinical response variables is an issue of continuing debate. Early studies showed an inverse relationship between initial serum haemagglutinin inhibition (HI) titre (by natural infection or vaccination) and postvaccination seroresponse [101] or susceptibility to infection [122], leading to the assumption of a correlate for protection.

Only a few studies addressed serological and clinical parameters [23, 33, 56, 63-65, 76, 102]. Of these studies, only one has shown a positive correlation between postvaccination serum antibody titres and the occurrence of laboratory confirmed influenza infections in elderly COPD patients [56]. Low seroprotection rates did not always correspond to a low clinical protection [64, 65, 76, 102] and revaccination has shown to increase the protection achieved by influenza vaccination [63, 65, 89, 92, 95]. Also in institutionalised elderly, despite a variable and sometimes limited seroresponsiveness, annual vaccination resulted in low numbers of symptomatic infections [61, 102]. In contrast, in 2 other studies in nursing home residents, vaccination did not exert a significant protection against influenza like illness [74] or mortality [33] during an aggressive and mismatched epidemic (1989-1990), despite seroprotective titres. As in the latter study [33] the non-vaccinated controls also showed a significant increase in seroprotection rates, one can not exclude re-infection by circulating wild virus in this institutionalised population.

Comment

The value of serological endpoints as a surrogate variable for clinical protection remains undetermined due to a surprising lack of studies correlating both serological and clinical endpoints. The main question that remains is why clinical protection sustains and apparently improves in elderly with compliance to annual vaccination when it is not reflected in seroresponse following repeated vaccination. The studies reviewed here do not support a straightforward correlation between serological parameters and clinical parameters.

First, there is the interpretation of the immune response following vaccination. The heterogeneity observed between the studies might very well reflect their intrinsic variability due to differences in study populations, and / or differences in responsiveness of individual strains [41], and also between laboratory variability of the haemagglutination inhibition (HI) assay [124]. The use of other methods to detect antibodies, or to characterise the immunity to influenza virus epitopes (from vaccine or infection) may be more sensitive than the classic HI assay [46, 56].

Revaccination appears to have a detrimental effect on seroresponse, but not on clinical protection, which might be a result of improved cellular immunity. Age-associated immune senescence has been promoted as a reason for revaccination [125]. Expanding T cell repertoire recognition for heterologous influenza strains as a result of revaccination might be essential for sustained protection against influenza in elderly. Specific cellular immunological parameters might, for example, provide useful information with regard to defining "risk categories" for impaired immune responsiveness [16, 126]. More detailed immune characterisation following influenza vaccination might allow for an understanding of clinical unpredictability of influenza epidemics, which may lead to negative results in one year but positive results in the other year [73, 82, 83], possibly by inhibiting interactions between vaccine strains and circulating strains [103, 104, 127]. More insight into these mechanisms may also lead to new design options for annual strain selection [105].

The conduct of outcome studies in large computerised databases allows for increasingly valid data over a wide variety of clinically important outcomes and covering several epidemic seasons [77-79, 81-83, 88, 89].

In conclusion, as clinical and serological outcomes following repeated exposure to influenza vaccine appear to diverge, it seems necessary to search for other, more reliable surrogate parameters. A decreased number of B lymphocytes in elderly, impairment of expansion [19, 53], inability to respond to new epitopes in presence of high baseline HI titres [16, 103] and age related differences in IgG subclass responses [32] have been reported as cause of the inability to mount adequate humoral responses after revaccination.

The importance of knowing how protection against influenza is induced by different components of the immune system, including immunological factors responsible for heterotypic and heterosubtypic protection and specifically the role of (cytotoxic) cellular immunity was recently reported [109]. The recommendations of this meeting support the view that claims with regard to clinical benefit on serological trials only are insufficient [52, 128].

References

1. Barker, W.H. and J.P. Mullooly, *Pneumonia and influenza deaths during epidemics: implications for prevention*. Arch Intern Med, 1982. 142(1): p. 85-9.
2. Thompson, W.W., et al., *Mortality associated with influenza and respiratory syncytial virus in the United States*. JAMA, 2003. 289(2): p. 179-86.
3. Fleming, D.M., *The contribution of influenza to combined acute respiratory infections, hospital admissions, and deaths in winter*. Commun Dis Public Health, 2000. 3(1): p. 32-8.
4. Sprenger, M.J., et al., *Impact of influenza on mortality in relation to age and underlying disease, 1967-1989*. Int J Epidemiol, 1993. 22(2): p. 334-40.
5. Neuzil, K.M., et al., *Winter respiratory viruses and health care use: a population-based study in the northwest United States*. Clin Infect Dis, 2003. 37(2): p. 201-7.
6. Widdicombe, J. and K. S., *Acute cough in the elderly: aetiology, diagnosis and therapy*. Drugs Aging, 2004. 21(4): p. 243-58.
7. Macfarlane, J., et al., *Prospective study of the incidence, aetiology and outcome of adult lower respiratory tract illness in the community*. Thorax, 2001. 56(2): p. 109-14.
8. Nicholson, K.G., et al., *Acute viral infections of upper respiratory tract in elderly people living in the community: comparative, prospective, population based study of disease burden*. BMJ, 1997. 315(7115): p. 1060-4.
9. Connolly, A.M., et al., *What are the complications of influenza and can they be prevented? Experience from the 1989 epidemic of H3N2 influenza A in general practice*. BMJ, 1993. 306(6890): p. 1452-4.
10. Barker, W.H. and J.P. Mullooly, *Impact of epidemic type A influenza in a defined adult population*. Am J Epidemiol, 1980. 112(6): p. 798-811.
11. Barker, W.H., et al., *Communitywide laboratory-based influenza surveillance focused on older persons, 1989-1992*. Am J Prev Med, 1995. 11(3): p. 149-55.
12. McBean, A.M., J.D. Babish, and J.L. Warren, *The impact and cost of influenza in the elderly*. Arch Intern Med, 1993. 153(18): p. 2105-11.
13. Carrat, F. and A.J. Valleron, *Influenza mortality among the elderly in France, 1980-90: how many deaths may have been avoided through vaccination?* J Epidemiol Community Health, 1995. 49(4): p. 419-25.
14. Carrat, F., et al., *Influenza burden of illness: estimates from a national prospective survey of household contacts in France*. Arch Intern Med, 2002. 162(16): p. 1842-8.
15. Musher, D., *How contagious are common respiratory tract infections?* N Engl J Med, 2003. 348: p. 1256-66.
16. Goronzy, J.J., et al., *Value of immunological markers in predicting responsiveness to influenza vaccination in elderly individuals*. J Virol, 2001. 75(24): p. 12182-7.
17. Clancy, R.L., *Mucosal vaccines for the prevention of influenza*. Drugs, 1995. 50(4): p. 587-94.
18. McElhaney, J.E., et al., *Split-virus influenza vaccines: do they provide adequate immunity in the elderly?* J Gerontol, 1994. 49(2): p. M37-43.
19. Glathe, H., S. Bigl, and A. Grosche, *Comparison of humoral immune responses to trivalent influenza split vaccine in young, middle-aged and elderly people*. Vaccine, 1993. 11(7): p. 702-5.
20. de Bruijn, I.A., et al., *Annually repeated influenza vaccination improves humoral responses to several influenza virus strains in healthy elderly*. Vaccine, 1997. 15(12-13): p. 1323-9.
21. Kang, I., et al., *Age-associated change in the frequency of memory CD4+ T cells impairs long term CD4+ T cell responses to influenza vaccine*. J.Immunol., 2004. 173: p. 673-81.

22. Huang, Y.P., et al., *The relationship between influenza vaccine-induced specific antibody responses and vaccine-induced nonspecific autoantibody responses in healthy older women*. J Gerontol, 1992. 47(2): p. M50-5.
23. Beyer, W.E., et al., *Protection against influenza after annually repeated vaccination: a meta-analysis of serologic and field studies*. Arch Intern Med, 1999. 159(2): p. 182-8.
24. Glathe, H. and W. Lange, *Influenza vaccination in older patients. Immunogenicity, epidemiology and available agents*. Drugs Aging, 1995. 6(5): p. 368-87.
25. Zei, T., M. Neri, and A.M. Iorio, *Immunogenicity of trivalent subunit and split influenza vaccines (1989- 90 winter season) in volunteers of different groups of age*. Vaccine, 1991. 9(9): p. 613-7.
26. Brydak, L.B., et al., *Immune response to influenza vaccination in an elderly population*. J Clin Immunol, 2003. 23(3): p. 214-22.
27. McElhaney, J.E., et al., *The effect of influenza vaccination on IL2 production in healthy elderly: implications for current vaccination practices*. J Gerontol, 1992. 47(1): p. M3-8.
28. McElhaney, J.E., et al., *Antibody response to whole-virus and split-virus influenza vaccines in successful ageing*. Vaccine, 1993. 11(10): p. 1055-60.
29. McElhaney, J.E., et al., *Granzyme B: a marker of risk for influenza in institutionalized older adults*. Vaccine, 2001. 19(27): p. 3744-51.
30. McElhaney, J.E., et al., *Vaccine-related determinants of the interleukin-2 response to influenza vaccination in healthy young and elderly adults*. Vaccine, 1995. 13(1): p. 6-10.
31. Powers, D.C. and R.B. Belshe, *Effect of age on cytotoxic T lymphocyte memory as well as serum and local antibody responses elicited by inactivated influenza virus vaccine*. J Infect Dis, 1993. 167(3): p. 584-92.
32. Powers, D.C., *Effect of age on serum immunoglobulin G subclass antibody responses to inactivated influenza virus vaccine*. J Med Virol, 1994. 43(1): p. 57-61.
33. Provinciali, M., et al., *Impaired antibody response to influenza vaccine in institutionalized elderly*. Ann N Y Acad Sci, 1994. 717: p. 307-14.
34. Remarque, E.J., et al., *Correlation between the antibody response to influenza vaccine and helper T cell subsets in healthy aging*. Vaccine, 1996. 14(2): p. 127-30.
35. Deng, Y., et al., *Age-related impaired type 1 T cell responses to influenza: reduced activation ex vivo, decreased expansion in CTL culture in vitro, and blunted response to influenza vaccination in vivo in the elderly*. J Immunol, 2004. 172(6): p. 3437-46.
36. Kohut, M.L., et al., *Exercise and psychosocial factors modulate immunity to influenza vaccine in elderly individuals*. J Gerontol A Biol Sci Med Sci, 2002. 57(9): p. M557-62.
37. Carson, P., et al., *Immune function and vaccine responses in healthy advanced elderly persons*. Arch.Intern.Med, 2000(160): p. 2017-24.
38. Gardner, E.M., et al., *Characterization of antibody responses to annual influenza vaccination over four years in a healthy elderly population*. Vaccine, 2001. 19(32): p. 4610-7.
39. Beyer, W.E., et al., *Effects of repeated annual influenza vaccination on vaccine sero- response in young and elderly adults*. Vaccine, 1996. 14(14): p. 1331-9.
40. Remarque, E.J., et al., *Improvement of the immunoglobulin subclass response to influenza vaccine in elderly nursing-home residents by the use of high-dose vaccines*. Vaccine, 1993. 11(6): p. 649-54.
41. Palache, A.M., et al., *Antibody response after influenza immunization with various vaccine doses: a double-blind, placebo-controlled, multi-centre, dose-response study in elderly nursing-home residents and young volunteers*. Vaccine, 1993. 11(1): p. 3-9.
42. Palache, A.M., et al., *Influenza vaccines: the effect of vaccine dose on antibody response in primed populations during the ongoing interpandemic period. A review of the literature*. Vaccine, 1993. 11(9): p. 892-908.

43. Roos-Van Eijndhoven, D.G., et al., *Randomized controlled trial of seroresponses to double dose and booster influenza vaccination in frail elderly subjects*. J Med Virol, 2001. 63(4): p. 293-8.
44. Powers, D.C., *Increased immunogenicity of inactivated influenza virus vaccine containing purified surface antigen compared with whole virus in elderly women*. Clin Diagn Lab Immunol, 1994. 1(1): p. 16-20.
45. Gross, P.A., et al., *Immunization of elderly people with two doses of influenza vaccine*. J Clin Microbiol, 1987. 25(9): p. 1763-5.
46. Powers, D.C., et al., *In elderly persons live attenuated influenza A virus vaccines do not offer an advantage over inactivated virus vaccine in inducing serum or secretory antibodies or local immunologic memory*. J Clin Microbiol, 1991. 29(3): p. 498-505.
47. Buxton, J.A., et al., *Influenza revaccination of elderly travelers: antibody response to single influenza vaccination and revaccination at 12 weeks*. J Infect Dis, 2001. 184(2): p. 188-91.
48. Wongsurakiat, P., et al., *Acute Respiratory Illness in Patients With COPD and the Effectiveness of Influenza Vaccination: A Randomized Controlled Study*. Chest, 2004. 125(6): p. 2011-2020.
49. Vogtlander, N.P., et al., *Impaired response rates, but satisfying protection rates to influenza vaccination in dialysis patients*. Vaccine, 2004. 22(17-18): p. 2199-201.
50. Muszkat, M., et al., *Local and systemic immune response in nursing-home elderly following intranasal or intramuscular immunization with inactivated influenza vaccine*. Vaccine, 2003. 21(11-12): p. 1180-6.
51. Squarcione, S., et al., *Comparison of the reactogenicity and immunogenicity of a split and a subunit-adjuvanted influenza vaccine in elderly subjects*. Vaccine, 2003. 21(11-12): p. 1268-74.
52. Frech, S., et al., *Improved immune responses to influenza vaccination in the elderly using an immunostimulant patch*. Vaccine, 2005. 23: p. 946-50.
53. Ben-Yehuda, A., et al., *The influence of sequential annual vaccination and of DHEA administration on the efficacy of the immune response to influenza vaccine in the elderly*. Mech Ageing Dev, 1998. 102(2-3): p. 299-306.
54. Katz, J.M., et al., *Immunity to influenza: the challenges of protecting an aging population*. Immunol Res, 2004. 29(1-3): p. 113-24.
55. Gorse, G.J., et al., *Influenza virus vaccination of patients with chronic lung disease*. Chest, 1997. 112(5): p. 1221-33.
56. Gorse, G.J., et al., *Immunity to influenza in older adults with chronic obstructive pulmonary disease*. J Infect Dis, 2004. 190(1): p. 11-9.
57. Dean, H. and C. D, *Epidermal powder immunization against influenza*. Vaccine, 2004. 23: p. 681-6.
58. Hoskins, T.W., et al., *Assessment of inactivated influenza-A vaccine after three outbreaks of influenza A at Christ's Hospital*. Lancet, 1979. 1(8106): p. 33-5.
59. *Committee for Proprietary Medicinal Products (CHMP). Note for Guidance on Harmonisation of Requirements for Influenza Vaccines*. (CHMP/BWP/214/96), (adopted March 1997).
60. Palache, A., I. de Bruyn, and J. Nauta, *Influenza immunisation. Part II: Fifteen years'experience with a subunit vaccine*. J.Clin Res, 1999(2): p. 132-9.
61. Nicholson, K.G., et al., *Immunogenicity of inactivated influenza vaccine in residential homes for elderly people*. Age Ageing, 1992. 21(3): p. 182-8.
62. Beyer, W.E., et al., *Seroprotection rate, mean fold increase, seroconversion rate: which parameter adequately expresses seroresponse to influenza vaccination?* Virus Res, 2004. 103(1-2): p. 125-32.
63. Howells, C.H., et al., *Influenza vaccination and mortality from bronchopneumonia in the elderly*. Lancet, 1975. 1(7903): p. 381-3.

64. Govaert, T.M., et al., *Immune response to influenza vaccination of elderly people. A randomized double-blind placebo-controlled trial.* Vaccine, 1994. 12(13): p. 1185-9.
65. Govaert, T.M., et al., *The efficacy of influenza vaccination in elderly individuals. A randomized double-blind placebo-controlled trial.* JAMA, 1994. 272(21): p. 1661-5.
66. Pyrhonen, S., J. Suni, and M. Romo, *Clinical trial of a subunit influenza vaccine.* Scand J Infect Dis, 1981. 13(2): p. 95-9.
67. Feery, B.J., M.G. Evered, and E.I. Morrison, *Different protection rates in various groups of volunteers given subunit influenza virus vaccine in 1976.* J Infect Dis, 1979. 139(2): p. 237-41.
68. Wongsurakiat, P., et al., *Economic evaluation of influenza vaccination in Thai chronic obstructive pulmonary disease patients.* J Med Assoc Thai, 2003. 86(6): p. 497-508.
69. Patriarca, P.A., et al., *Efficacy of influenza vaccine in nursing homes. Reduction in illness and complications during an influenza A (H3N2) epidemic.* JAMA, 1985. 253(8): p. 1136-9.
70. Foster, D.A., et al., *Influenza vaccine effectiveness in preventing hospitalization for pneumonia in the elderly.* Am J Epidemiol, 1992. 136(3): p. 296-307.
71. Saah, A.J., et al., *Influenza vaccine and pneumonia mortality in a nursing home population.* Arch Intern Med, 1986. 146(12): p. 2353-7.
72. Lin Taylor, J. and e. al., *Nursing home outbreak of influenza (H3N2): evaluation of vaccine efficacy and influenza case definitions.* Infect Control Hosp Epidemiol, 1992. 13: p. 93-7.
73. Barker, W.H. and J.P. Mullooly, *Influenza vaccination of elderly persons. Reduction in pneumonia and influenza hospitalizations and deaths.* JAMA, 1980. 244(22): p. 2547-9.
74. Dindinaud, G., et al., *Influenza epidemic among a community of elderly people in spite of vaccination.* Eur J Epidemiol, 1993. 9(6): p. 667-70.
75. Morens, D.M. and V.M. Rash, *Lessons from a nursing home outbreak of influenza A.* Infect Control Hosp Epidemiol, 1995. 16(5): p. 275-80.
76. Odelin, M.F., et al., *Role of influenza vaccination in the elderly during an epidemic of A/H1N1 virus in 1988-1989: clinical and serological data.* Gerontology, 1993. 39(2): p. 109-16.
77. Nichol, K.L., *Complications of influenza and benefits of vaccination.* Vaccine, 1999. 17 Suppl 1: p. S47-52.
78. Nichol, K.L., et al., *The efficacy and cost effectiveness of vaccination against influenza among elderly persons living in the community.* N Engl J Med, 1994. 331(12): p. 778-84.
79. Nichol, K.L., et al., *Effectiveness of influenza vaccine in the elderly.* Gerontology, 1996. 42(5): p. 274-9.
80. Nichol, K.L., et al., *Influenza vaccination and reduction in hospitalizations for cardiac disease and stroke among the elderly.* N Engl J Med, 2003. 348(14): p. 1322-32.
81. Hak, E., et al., *Influence of high-risk medical conditions on the effectiveness of influenza vaccination among elderly members of 3 large managed-care organizations.* Clin Infect Dis, 2002. 35(4): p. 370-7.
82. Mullooly, J.P., et al., *Influenza vaccination programs for elderly persons: cost-effectiveness in a health maintenance organization.* Ann Intern Med, 1994. 121(12): p. 947-52.
83. Nordin, J., et al., *Influenza vaccine effectiveness in preventing hospitalizations and deaths in persons 65 years or older in Minnesota, New York, and Oregon: data from 3 health plans.* J Infect Dis, 2001. 184(6): p. 665-70.
84. Fedson, D.S., et al., *Clinical effectiveness of influenza vaccination in Manitoba.* JAMA, 1993. 270(16): p. 1956-61.
85. Wang, S., et al., *Economic evaluation of vaccination against influenza in the elderly: n experience from a population-based influenza vaccination program in Taiwan.* Vaccine, 2005. 23: p. 1973-80.

86. Christenson, B., et al., *Effects of a large-scale intervention with influenza and 23-valent pneumococcal vaccines in adults aged 65 years or older: a prospective study*. Lancet, 2001. 357(9261): p. 1008-11.
87. Nicholson, K.G., J. Kent, and V. Hammersley, *Influenza A among community-dwelling elderly persons in Leicestershire during winter 1993-4; cigarette smoking as a risk factor and the efficacy of influenza vaccination*. Epidemiol Infect, 1999. 123(1): p. 103-8.
88. Armstrong, B., et al., *Effect of influenza vaccination on excess death occurring during periods of high circulation of influenza: cohort study in elderly people*. BMJ, 2004. doi:10.1136/BMJ.38198.594109.AE((published 15 August 2004)).
89. Voordouw, A.C.G., et al., *Annual revaccination against influenza and mortality risk in community dwelling elderly persons*. JAMA, 2004. 292(17): p. 2089-95.
90. Voordouw, B.C., et al., *Influenza vaccination in community-dwelling elderly: impact on mortality and influenza-associated morbidity*. Arch Intern Med, 2003. 163(9): p. 1089-94.
91. Carrat, F., et al., *Field investigation of influenza vaccine effectiveness on morbidity*. Vaccine, 1998. 16(9-10): p. 893-8.
92. Fleming, D.M., et al., *Study of the effectiveness of influenza vaccination in the elderly in the epidemic of 1989-90 using a general practice database*. Epidemiol Infect, 1995. 115(3): p. 581-9.
93. Mangtani, P., et al., *A cohort study of the effectiveness of influenza vaccine in older people, performed using the United kingdom general practice research database*. J Infect Dis, 2004. 190(1): p. 1-10.
94. Nichol, K.L., L. Baken, and A. Nelson, *Relation between influenza vaccination and outpatient visits, hospitalization, and mortality in elderly persons with chronic lung disease*. Ann Intern Med, 1999. 130(5): p. 397-403.
95. Colquhoun, A.J., et al., *Effectiveness of influenza vaccine in reducing hospital admissions in people with diabetes*. Epidemiol Infect, 1997. 119(3): p. 335-41.
96. Kubiet, M.A., et al., *Serum antibody response to influenza vaccine in pulmonary patients receiving corticosteroids*. Chest, 1996. 110(2): p. 367-70.
97. Vu, T., et al., *A meta-analysis of effectiveness of influenza vaccine in persons aged 65 years and over living in the community*. Vaccine, 2002. 20(13-14): p. 1831-6.
98. Gross, P.A., et al., *The efficacy of influenza vaccine in elderly persons. A meta-analysis and review of the literature*. Ann Intern Med, 1995. 123(7): p. 518-27.
99. Nichol, K.L., *The efficacy, effectiveness and cost-effectiveness of inactivated influenza virus vaccines*. Vaccine, 2003. 21(16): p. 1769-75.
100. Hannoun, C., F. Megas, and J. Piercy, *Immunogenicity and protective efficacy of influenza vaccination*. Virus Res, 2004. 103(1-2): p. 133-8.
101. Hobson, D., et al., *The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses*. J Hyg (Lond), 1972. 70(4): p. 767-77.
102. Odelin, M.F., et al., *Temporal surveillance of the humoral immunity against influenza vaccine in the elderly over 9 consecutive years*. Gerontology, 2003. 49(4): p. 233-9.
103. Smith, D.J., et al., *Variable efficacy of repeated annual influenza vaccination*. Proc Natl Acad Sci U S A, 1999. 96(24): p. 14001-6.
104. Smith, D.J., et al., *Mapping of the antigenic and genetic evolution of influenza virus*. Science, 2004. 305: p. 371-376.
105. Munoz, E. and M. Deem, *Epitope analysis for influenza vaccine design*. Vaccine, 2005. 23: p. 1144-8.
106. DerSimonian, R. and N. Laird, *Meta-analysis in clinical trials*. Contr.Clin.Trials, 1986(7): p. 177-88.

107. Iorio, R.M., et al., *Structural and functional relationship between the receptor recognition and neuraminidase activities of the Newcastle disease virus hemagglutinin-neuraminidase protein: receptor recognition is dependent on neuraminidase activity*. J Virol, 2001. 75(4): p. 1918-27.
108. Rastogi, S., et al., *Time to peak serum antibody response to influenza vaccine*. Clin Diagn Lab Immunol, 1995. 2(1): p. 120-1.
109. Cassetti, M., et al., *Report of meeting on the development of influenza vaccines with broad spectrum and long-lasting immune responses, World Health Organization, Geneva, Switzerland, 26-27 February 2004*. Vaccine, 2005. 23(13): p. 1529-1533.
110. Walsh, E.E., C. Cox, and A.R. Falsey, *Clinical features of influenza A virus infection in older hospitalized persons*. J Am Geriatr Soc, 2002. 50(9): p. 1498-503.
111. Deguchi, Y., Y. Takasugi, and K. Tatara, *Efficacy of influenza vaccine in the elderly in welfare nursing homes: reduction in risks of mortality and morbidity during an influenza A (H3N2) epidemic*. J Med Microbiol, 2000. 49(6): p. 553-6.
112. Allsup, S., et al., *In influenza vacciantion cost effective for healthy people etween 65 and 74 years? A randomised controlled trial*. Vaccine, 2004. 23: p. 639-45.
113. Ebell, M.H., L.L. White, and T. Casault, *A systematic review of the history and physical examination to diagnose influenza*. J Am Board Fam Pract, 2004. 17(1): p. 1-5.
114. Zambon, M., et al., *Diagnosis of influenza in the community: relationship of clinical diagnosis to confirmed virological, serologic, or molecular detection of influenza*. Arch Intern Med, 2001. 161(17): p. 2116-22.
115. Nicholson, K.G., *Immunisation against influenza among people aged over 65 living at home in Leicestershire during winter 1991-2*. BMJ, 1993. 306(6883): p. 974-6.
116. Puig-Barbera, J., et al., *Reduction in hospital admissions for pneumonia in non-institutionalised elderly people as a result of influenza vaccination: a case-control study in Spain*. J Epidemiol Community Health, 1997. 51(5): p. 526-30.
117. Arden, N., A.S. Monto, and S.E. Ohmit, *Vaccine use and the risk of outbreaks in a sample of nursing homes during an influenza epidemic*. Am J Public Health, 1995. 85(3): p. 399-401.
118. Nicholson, K.G., et al., *Acute upper respiratory tract viral illness and influenza immunization in homes for the elderly*. Epidemiol Infect, 1990. 105(3): p. 609-18.
119. Ahmed, A.E., K.G. Nicholson, and J.S. Nguyen-Van-Tam, *Reduction in mortality associated with influenza vaccine during 1989-90 epidemic*. Lancet, 1995. 346(8975): p. 591-5.
120. Keitel, W.A., T.R. Cate, and R.B. Couch, *Efficacy of sequential annual vaccination with inactivated influenza virus vaccine*. Am J Epidemiol, 1988. 127(2): p. 353-64.
121. Keitel, W.A., et al., *Efficacy of repeated annual immunization with inactivated influenza virus vaccines over a five year period*. Vaccine, 1997. 15(10): p. 1114-22.
122. Potter, C.W. and J.S. Oxford, *Determinants of immunity to influenza infection in man*. Br Med Bull, 1979. 35(1): p. 69-75.
123. Smith, C.B., et al., *Molecular epidemiology of influenza A(H3N2) virus reinfections*. J Infect Dis, 2002. 185(7): p. 980-5.
124. Wood, J.M., et al., *Comparison of influenza serological techniques by international collaborative study*. Vaccine, 1994. 12(2): p. 167-74.
125. de Jong, J.C., et al., *Mismatch between the 1997/1998 influenza vaccine and the major epidemic A(H3N2) virus strain as the cause of an inadequate vaccine-induced antibody response to this strain in the elderly*. J Med Virol, 2000. 61(1): p. 94-9.
126. Pawelec, G., et al., *Is human immunosenescence clinically relevant? Looking for "immunological risk phenotypes."* Trends Immunol., 2002. 23(7): p. 330-2.
127. Beyer, W.E., et al., *The plea against annual influenza vaccination? 'The Hoskins' Paradox' revisited*. Vaccine, 1998. 16(20): p. 1929-32.

128. De Bruijn, I.A., et al., *Virosomal influenza vaccine: a safe and effective influenza vaccine with high efficacy in elderly and subjects with low pre-vaccination antibody titers*. Virus Res, 2004. 103(1-2): p. 139-45.
129. Landi, F., et al., *Effects of influenza vaccination on mortality among frail, community-living elderly patients: an observational study*. Aging Clin Exp Res, 2003. 15(3): p. 254-8.

8

General Discussion

1 Background

Epidemics caused by influenza viruses are associated with considerable morbidity and mortality which increases with age, especially in patients with high-risk conditions [1, 2]. Therefore, many countries recommend annual influenza vaccination of elderly individuals and of adults and children with specific high risk conditions [3]. The composition of the influenza vaccine is determined yearly following recommendation by the World Health Organisation.

As in general the strain composition changes each year, influenza vaccines are relicensed annually, after having obtained an initial licensure based on a full preclinical and clinical dossier. Within the European Union, the annual relicensure requires some proof of immunogenicity and acceptable reactogenicity following vaccination. For this purpose, the marketing authorization holders perform small scale open label trials in a selected population of healthy adults and elderly. The requirements for these trials are described in the "Note for Guidance on Harmonisation of requirements for influenza vaccines" (CHMP/BWP/214/96) [4]. The objective of these trials is mainly to assess the immunogenicity of annual influenza vaccines (in case of new components) as a proxy for clinical protective efficacy. However, individuals are exposed, by infection or vaccination, to different influenza virus (sub)types and strains during their lifetime [5]. Moreover, ageing and health status may affect humoral responses to influenza viral antigens [6-14]. Consequently, it is questionable whether these annual update trials are adequate to answer this objective.

Clinical outcomes following influenza vaccination are mainly investigated in observational studies. The ability to detect a clinical benefit of vaccination with regard to the risk to develop influenza like illness also depends upon the availability of serological or virological methods to ascertain influenza infection [15-17]. The most common complication following influenza infection is acute bronchitis, which risk increases in elderly and in individuals with underlying chronic conditions [18]. Hospitalisation rates for pneumonia and influenza, acute bronchitis and chronic respiratory disease are significantly higher during influenza periods [19, 20]. In several studies the impact of vaccination on influenza, pneumonia or acute respiratory infections has been addressed. Generally, it seems that in individuals with underlying chronic disease vaccination is less effective than in individuals without such conditions [21-28].

The ability of influenza vaccination to reduce mortality risk has been reported quite consistently in community dwelling elderly [21, 23, 24, 29-32]. More than 80% of all pneumonia and influenza related deaths are reported to occur in elderly aged ≥65 years [33].

According to the Statistics Netherlands (CBS), influenza causes an excess mortality of more than 2000 persons per year. In the 2004-2005 season, 800 excess deaths were calculated despite a relatively mild epidemic activity [34].

This estimated impact of annual influenza epidemics on morbidity and mortality in elderly, and the assumed (cost-) effectiveness of influenza vaccination, has been the basis for implementing nationwide influenza vaccination programs for elderly [3]. Annual revaccination has been proposed as a strategy to compensate for the decrease in effectiveness in high risk individuals [6,25, 31, 35-39]. Although in a meta-analysis no specific advantage of revaccination over single vaccination was observed in the pooled analysis, 7 out of 10 field trials supported sustained protection against laboratory confirmed influenza like illness upon revaccination [40]. In institutionalised elderly, a positive association between vaccination coverage, revaccination status, and/or antigenic match between circulating strain and vaccine strain and vaccine effectiveness was observed [41-45].

But, although seasonal epidemiological surveillance of influenza infections is standard practice, and it is recommended to vaccinate all elderly of 65 years and older, effectiveness of annual influenza vaccination in relation to vaccination history and health status of community dwelling elderly has not systematically been evaluated.

This chapter summarises the most important findings from the studies performed to address the question of the benefit of annual revaccination against influenza in community dwelling elderly. The methodological considerations underlying these studies are discussed. Finally, some ideas and suggestions are given for future research to further clarify the impact of influenza revaccination. Also, some alternatives are suggested for the present practice of serological update trials for annual relicensure of influenza vaccines.

2 Main findings

2.1 *Impact of influenza vaccination on mortality, pneumonia and influenza like illness*

The influenza season of 1996-1997 was of mild to moderate severity, with a peak activity of influenza like illness of 28.8 per 10,000 persons observed in week 4 of 1997 and a distribution in elderly similar to the general population [25]. There was a good match between vaccine strain and the predominant circulating strainA/H3N2/Nanchang/933/95. In this epidemic period, we investigated the effectiveness of influenza vaccination in a cohort of community dwelling elderly aged 65 years or older using the IPCI database [25].

Influenza vaccination effectively prevented mortality and morbidity (RR, 0.72; $CI_{95\%}$: 0.60-0.87), although in individuals with co-morbidity, influenza vaccination failed to show a protective effect against pneumonia. Influenza infections were identified by the ICPC-code R80.0 ("proven infection without pneumonia"). Influenza infections decreased by 52% in the vaccinated population ($CI_{95\%}$: 9-74%), although this figure was based upon 48 cases only (16 in the vaccinated cohort and 32 in the control cohort). Most of excess mortality due to influenza and/or pneumonia is attributed to elderly with high-risk conditions [46]. In our study, influenza vaccination reduced mortality risk by 24% ($CI_{95\%}$: 3-40%) in the total population. This risk reduction was 33% ($CI_{95\%}$: 6-54%) in elderly with co-morbidity. On the contrary, however, influenza vaccination had no significant benefit with regard to pneumonia in the total population (RR, 0.77, $CI_{95\%}$: 0.55-1.07]). However, in the population without co-morbidity vaccination significantly reduced the risk by 44% ($CI_{95\%}$: 11-65%). We made a similar observation in the revaccination study described in chapter 5.

We concluded that it might be possible that the failure to detect a benefit of vaccination in elderly with chronic underlying disease may result from the fact that the subjects die before developing clinical pneumonia, or that the pneumonia is not recognized, because symptoms are less prominent.

2.2 *Revaccination and mortality risk*

In this first study, we did a preliminary assessment of annual revaccination. In the population with a vaccination history the impact of vaccination on mortality risk was more prominent. But numbers were too small in the different subpopulations to allow for appropriate conclusions.

Following the first study, the issue of revaccination was further explored. Although annual influenza revaccination has been described in the literature as a strategy to increase vaccination effectiveness [6, 31, 38, 39], the benefit was not always obvious from clinical studies. In institutionalised elderly, annual revaccination as well as vaccination coverage showed to be the most consistent contributors to survival [42, 44]. But there were no such data for community dwelling elderly. With regard to serologically or virologically confirmed influenza, revaccination had no additional impact on the risk in both community dwelling elderly [35] and in healthy adults (aged 30 to 60) [47, 48]. However, in both cases, first vaccination and revaccination resulted in better protection against influenza virus infection compared to placebo. These findings were confirmed in a meta-analysis [38]. The possible association between mortality and revaccination status in elderly was studied in only one case-control study [31]. This study indeed showed that a previous vaccination significantly increased vaccine

effectiveness in the next season. However, the population included both community dwelling and institutionalised individuals.

From the first study we knew that it was possible to obtain detailed information on vaccination status for each individual. This gave the opportunity to assess the overall and epidemic effectiveness of annual influenza vaccinations, including the effect of individual revaccinations. However, a greater sample size was required to allow valid conclusions. Therefore, the effect of annual revaccination on all cause mortality was investigated over the period 1996-2002.

The most important finding was that revaccination was associated with a significantly reduced mortality risk compared to both the non-vaccinated reference group but also compared to subjects who had received only one vaccination. During the epidemic periods, the apparent benefit of revaccination compared to a first vaccination was even stronger. The effect was apparent in both healthy elderly and in those with underlying chronic disease. Remarkable was the finding that revaccination mainly resulted in a significant mortality reduction among those aged 70 years and older, which seemed to reflect differences in age related causes of death, and a lower baseline risk to die at younger age. And indeed the subjects for whom the cause of death was classified as "infections" or "natural causes" had the highest mean age. For these 2 causes of death the effect of vaccination was strongest.

Another main finding was the fact that interruption of yearly influenza vaccination was associated with a significantly increased mortality, but after restarting vaccination mortality risk reduced again to a revaccination status level. Absence of sustained protection from vaccination was studied as explanation for the observed risk increase. We observed that in subjects who interrupted vaccination for 2 or more consecutive years mortality risk seemed to increase even more. Although epidemiological studies do not allow for assessment of causal relations, one can not exclude that these finding support the relevance of complying to annual revaccination, especially at older age.

To compensate for the age-related decline in antibody response, or immunosenescence, [10, 39, 49-53], the maintenance of cross-reactive immunity conferred by T cell may be especially important. As cellular immunity is also affected by age [6-12, 54-56], annual revaccination might provide the "boosting" effect necessary to sustain clinical protection and to reduce variations in protective efficacy. From studies in nursing homes, we already learned that herd immunity was associated with a lower dependency on the antigenic match between vaccine and virus [14-16]. In these settings, most individuals are also vaccinated each year.

2.3 *Revaccination and sudden cardiac death*

Cardiovascular morbidity and mortality, particularly acute myocardial infarction, stroke and sudden cardiac death show seasonal variation, with peak incidences in winter months [23, 37, 57-59]. Influenza activity has been suggested as an explanation for the winter peak of myocardial infarction [60] and it was suggested that Sudden Cardiac Death (SCD) may be a complication following influenza virus infection [57, 61, 62]. Consequently, it might be hypothesized that influenza vaccination protects against SCD.

Indeed, in our study revaccination was associated with a decreased risk of sudden cardiac death. However, a first vaccination showed a tendency towards an increased higher risk (HR, 1.50, $CI_{95\%}$: 0.98-2.32), which became significant for the subpopulation without co-morbidity (HR, 2.44, $CI_{95\%}$:1.00-5.98). However, precision was low. Recent findings did not show an association between vaccination and an increased risk of SCD [63], and it is not clear how the influenza vaccination could increase the risk of SCD after a first vaccination, but not after revaccination.

A remarkable finding was the important association between those refusing vaccination and the outcome. In the overall population, those refusing vaccination were at a 52% ($CI_{95\%}$: 19-74%) lower risk of SCD compared to the overall non-vaccinated reference group. The belief to be in good health is an already reported reason for vaccination refusal [3].

2.4 *Revaccination and the risk of lower respiratory tract infections*

As mentioned in paragraph 2.1, the benefit of revaccination is most obvious in its association with reduction of mortality. For serologically or virologically confirmed influenza infection, no additional impact of revaccination was observed in both community dwelling elderly [35] and in healthy adults (aged 30 to 60) [47, 48]. However, during winter periods, up to 30% of elderly may experience acute respiratory infections of which up to 20% are attributed to influenza virus [16, 64-66].

Respiratory complications following influenza infection include acute bronchitis, pneumonia and exacerbations of chronic bronchitis or asthma [67]. In general practice, acute bronchitis following influenza infection is the most common complication and the risk increases in elderly and in those with underlying conditions [18].

We found no published data on the effectiveness of influenza vaccination against LRTI in community dwelling elderly. Therefore, we investigated the risk of lower respiratory tract infections (LRTI) following annual revaccination in our cohort of community dwelling elderly [24].

In the overall population, the study did support a benefit of annual influenza vaccination for clinically established LRTI or pneumonia, when analysed for the epidemic period, but only for those without recorded comorbidity. For patients hospitalised with pneumonia, influenza vaccination was already associated with a significant protection [21, 68-70].

Also in this study it was apparent that adjustment for known risk factors did not fully appreciate other population factors underlying the risks to develop LRTI or pneumonia.

Previous studies that addressed health status as a factor in establishing vaccine effectiveness in LRTI or pneumonia, also reported a stronger benefit of the relatively healthier subpopulation [26, 71], although a stronger benefit for the population with underlying disease has also been reported [36].

A final interesting finding in this study was the importance of the characteristics of the epidemics. Only in years with moderate epidemic activity revaccination was associated with a substantially lower risk of LRTI or pneumonia compared to a first vaccination. Possibly the attack rates during the epidemic seasons 1996-1997 and 1999-2000 were just sufficient to observe an impact of revaccination. Epidemic characteristics are described in many studies but not often do the data allow for epidemic specific subanalyses.

2.5 *Immunogenicity of influenza vaccines*

Within the European Union, influenza vaccines are relicensed annually following determination of their composition by the World Health Organisation. This relicensure requires some clinical data to support the immunogenicity and reactogenicity of the vaccine to be administered in the upcoming season. These requirements are laid down in the "Note for Guidance on Harmonisation of requirements for influenza vaccines" (CHMP/BWP/214/96) [4].

The annual update trials are analysed for seroprotection rate and seroresponse rate. In adults, the defined threshold titre for protection of 40 measured by haemagglutination inhibition (HI) assay may be a fair assumption [72], but it is not clear whether this also applies for elderly and it is never studied in children. Older studies indicated a threshold of 120 in elderly to protect against more severe outcomes such as pneumonia [39, 73].

The annual update trials are meant to evaluate the immunogenicity of annual influenza vaccines. Therefore it is important to assure that the responses which are measured indeed reflect the immunogenicity of the vaccine and not population characteristics such as age, health status and a widely variable exposition, by infection or vaccination, to different influenza viruses during ones' lifetime [5].

In 48 age-defined trials, which we controlled for health status, age and prevaccination titre, we observed that increasing pre-vaccination antibody levels significantly contributed to seroprotection. Furthermore, post-vaccination parameters were strongly dependent on age. However, adjustment for these factors may not be sufficient as it does not take into account that these population characteristics may be correlated. In addition to pre-vaccination titre and (unapparent) co-morbidity, the previous vaccinations may be dependent on age. But pre-vaccination titres also depend on previous vaccinations, health status etc. resulting in a complex pattern of interactions. Another complicating factor might be the observation that correlations between vaccine strains and circulating strains apparently play a more profound role in clinical performance of influenza vaccines than previously assumed, especially in those annually revaccinated [74, 75].

Also in our analysis, previous influenza vaccinations significantly affected pre-, but not post-vaccination titres. Impaired postvaccination seroprotection rate or seroresponse rate is inevitable in the presence of high baseline titres, and is mainly observed in those with a vaccination history. Several authors have hypothesized about this issue, including suggestions about a decreased pool of B lymphocytes and thus an impaired ability to expand [52, 76], inability to respond to new epitopes in presence of high baseline HI titres [49, 74], and age related differences in IgG subclass responses [56].

It is questionable whether for an inactivated vaccine with a mainly humoral driven response, serological data in elderly individuals, who are exposed to a wide variety of influenza strains during their life time, are likely to provide any reliable information with regard to clinical protection. Even with low postvaccination seroprotection rates in an elderly GP patient population, stronger clinical protection was achieved for those patients with a vaccination history [35]. As humoral driven responses do not necessary show a booster effect, it may indeed be more relevant to focus on the possible positive role of cellular immunity [35], as it may decrease variations in protective potential of a vaccine through sustained cross-protection [77].

2.6 *Serological responses as correlate for clinical protection*

Trying to understand serological trials and clinical responses in elderly individuals the main issue is why clinical protection seems to improve with annual revaccination whereas it is not reflected in seroresponsiveness. There is obviously no support for a straightforward correlation between serological and clinical parameters. The high baseline titres resulting in blurred post vaccination responses are not predictive of an impaired immune response. On the contrary, high baseline titres are usually found in

individuals with a vaccination history [5], and the presence of a vaccination history is associated with increased protection [24, 31].

A decline in humoral immunity does not necessarily imply a decline in cellular immunity and vice versa [10, 78]. Apparently a long history of exposure to influenza virus and./or vaccine leads to blurring of the humoral response, but not necessarily to an impaired protection. The efficacy of annual variations in influenza vaccines is measured by serological parameters and clinical outcomes are measured in observational studies. These different settings make it complex and studies addressing both clinical and serological parameters did not correlate the 2 outcomes. In the literature we found only 2 studies which presented both evaluable clinical and serological parameters [35, 79, 80].

Given the highly variable influenza history of elderly persons one should question the relevance of pursuing on serological studies following influenza vaccination without additional confirmation of other immunological or clinical parameters. It might be more useful to focus on cellular immune response as a proxy for (sustained) clinical protection following revaccination [49, 77, 81]. Further immunological characterisation following influenza vaccination, and modelling could also allow for an understanding of clinical unpredictability of influenza epidemics, which may lead to negative results in one year but not in another [26, 70, 82]. The availability of large computerised databases allow for increasingly valid research on a wide variety of clinically important outcomes over several epidemic seasons [21, 23-26, 30, 36, 42, 82, 83].

More insight into the relevant immunological parameters [49, 81] and virus to vaccine interactions [74, 75, 84] is necessary to improve the validity of the observational studies, which may result in better defined risk factors. The need for this has already been expressed, but was focussed on clinical parameters only [85, 86]. In addition to better defined case definitions, this may also contribute to more specific and valid outcomes [87]. This is not only important to better quantify effects of influenza revaccination in elderly, but also in adults and children with chronic underlying disease.

3 Methodological considerations

3.1 *Validity*

Whereas in experimental trials randomisation and blinding procedures often guarantee valid study results, the validity of the results in observational studies might be endangered by selection bias, information bias and confounding.

Selection bias

Selection bias is mainly an issue when inclusion of cases and controls depends to some extent on the exposure of interest. In this respect selection bias is most important in case control studies. However, also cohort studies may suffer from selection bias, if the exposure status depends upon the outcome of interest. In the cohort studies described in chapters 2-5, selection bias was considered unlikely, as in the Dutch health care system all individuals are designated to their own GP. Hence, all morbidity and mortality comes to the attention of the GP. As the computerized data collection is complete for all patients who consult their doctor, the chance of selection bias in these studies is negligible.

In the serology trials selection bias is possibly a more serious issue. These open label uncontrolled trials are especially prone to selection bias, e.g., for age to enhance the possibility of a positive outcome of interest. In the study described in chapter 6, we showed that over the follow up period the median age of the younger age category decreased substantially. The median age of the elderly trial population was relatively low throughout the study period.

Information bias

Information bias is a potential source of major error in cohort studies, as it depends upon the validity by which subjects are classified to their exposure and outcome [88].

In cohort studies, information bias may have occurred if the vaccination was not recorded or a (re)vaccination status was assigned to the wrong follow up period. However, it is likely that such misclassification is random because exposure is prospectively recorded without prior knowledge of the research hypothesis and before the outcome of interest occurred. A consequence of such random misclassification would be that our estimates were a conservative one and that the real protective effect might even be higher.

Misclassification of death as studied in chapters 2-4 is probably minimal since death rates in IPCI are in line with national data on mortality. Moreover, death is usually adequately registered at GP practices. Misclassification of lower respiratory tract infections as studied in chapter 5, may be a problem. Despite the fact that the availability of free text is a valuable tool in the validation process as it allows for controlling possible non-uniform application of ICPC codes by GPs, it does not enhance sensitivity of the used clinical criteria. Microbiological confirmation is important [16, 89] as respiratory tract infections are common in elderly, especially during winter months [89]. Causative organisms are identified in only a limited number of specimen

[66], and in elderly other viruses than influenza virus (e.g. RSV, rhinoviruses, corona viruses) are responsible [64, 89]. Also clinical symptoms of LRTI can be very variable in community dwelling elderly [66].

Misclassification of pneumonia, studied in chapters 2 and 5, might be a less important issue as all cases were confirmed radiologically and/or microbiologically. However, also for pneumonia, especially in elderly with an impaired clinical condition one can not exclude underreporting of pneumonia, because the patient died before developing a clinical picture of pneumonia, or pneumonia is not recognised, because as for LRTI, symptoms in elderly may be less prominent. It is possible that influenza infection, studied in chapter 2, was underestimated in the vaccine cohort because doctors were aware of the vaccination status of their patients.

Confounding

Confounding is a potential serious problem for all observational studies. In confounding the effect of the exposure under study on the outcome is mixed with a third factor. This third factor may cause the disease, but is also associated with the exposure, without being an intermediate step between exposure and outcome [90]. Important here is that the variable is also a risk factor for the disease in the non-exposed and is also associated with the exposure in the non-diseased. Adjustment for confounding is possible through restriction, matching, stratification and multivariate techniques. For influenza vaccine studies, confounding factors include age, vaccination history and underlying disease.

In the cohort studies described in chapters 2-5 confounding by indication was addressed in detail. In all cohort studies, we assumed confounding by indication since pre-existing chronic respiratory tract disease, cardiovascular tract disease, diabetes mellitus, malignancies (excluded in the SCD study) and chronic renal insufficiencies have an increased risk of death and are strongly advised to have an influenza vaccination.

With regard to health status, a perceived good health may be a reason for non-compliance to the influenza vaccination program [3]. Also in our studies, a substantial proportion refused vaccination for one or more years. But the differential effect of refusers on the outcome may well have been a result of residual confounding. In the revaccination study discussed in chapter 3, study subjects listed as vaccine refusers showed a more or less similar mortality risk as those receiving a first vaccination; however, they were at a significantly increased risk compared to the remaining reference group. For the outcomes SCD and LRTI, studied in chapter 4 and 5 respectively, the health status (health beliefs) of those refusing vaccination did have a substantial impact on the outcome. Especially in the LRTI study residual confounding is likely,

and has probably led to an underestimation of the true effect of influenza vaccination, which included acute exacerbation of chronic bronchitis. Other factors may have contributed even more significantly to the risk to develop LRTI, such as pollution, self treatment and the delivery of pneumococcal vaccination [89].

However, as residual confounding cannot be excluded, a poorer prognosis in subjects who were vaccinated means that we underestimated rather than overestimated the protective effect of annual revaccination.

Confounding factors in the serology trials are also age, vaccination history and health status. To adjust for age and vaccination history we used restriction and stratification. To adjust for health status we use restriction, by excluding all individuals for whom any comorbidity known to be associated with both an indication for vaccination and an outcome of interest was registered. However, this does mean that all vaccinated subjects were in good health. Only a limited number of studies reported individual data on comorbidity, none of these studies was performed before 1995.

3.2 *Generalisability*

The generalisability is important to extrapolate the results to the general population. This is an advantage of epidemiological research and a limitation of experimental trials.

The demographic characteristics in the IPCI database are in accordance with the formal national registry CBS (Statistics Netherlands). The vaccination coverage for IPCI is also in agreement with CBS figures. The vaccination coverage in IPCI varied between 64-74%. According to CBS in 2002 the vaccination coverage in elderly aged ≥ 65 years was 73.6%.

Also the IPCI database complies with EC guidelines on the use of data for medical research [91].

4 Conclusions and future research

Despite the fact that the first inactivated influenza vaccines were licensed in 1945 [92], today there are still a number of unsolved questions. These questions relate to the immunological sequelae following vaccination and their correlation to clinical protection. Research activities in this respect might take into consideration the following views.

1. Serological parameters do not simply correlate with clinical protection, especially since each vaccinated individual carries his or her personal infection and vaccination history. It is likely that annual revaccination is especially important to

maintain cellular immunity, which is necessary for cross-reactive immunity, thus reducing the risks associated with antigenic drift. The need for research in this direction was recently expressed at an expert meeting [77].

2. Evidence of the benefit of annual revaccination is not unambiguous. Observed variability of clinical effectiveness following revaccination has been explained by antigenic differences among the vaccine and epidemic strains [74, 75] or differential epitope susceptibility to new influenza antigens as a result of previous exposure to influenza antigens [84]. The availability of large computerised databases such as the IPCI or the GPRD database allow for prospectively generated extensive information on influenza vaccination and outcomes. Defining how to incorporate vaccination and /or infection history (i.e. "antigenic- history") into the risk adjustment of these observational datasets, might further clarify the issue of observed variability in clinical outcomes in different epidemic seasons. Ultimately, the result of such research might be a different attitude towards annual strain selection.

3. It seems that the time has come to reconsider the CHMP criteria and to study alternatives for annual update trials. For the purpose of such trials, a homogenous population should be selected in which the impact of disturbing population factors is minimal. The assessment criteria for such studies should be redefined. On the other hand, one might also conclude that with the availability of large computerised databases and more insight into the immunology, assessment of the in vitro potency is sufficient for annual strain variations. This assessment of in vitro potency is already common practice elsewhere.

References

1. Murray, C. and A. Lopez, *Mortality by cause for eight regions of the world: Global Burden of Disease Study*. Lancet, 1997. 349: p. 1269-76.
2. Sprenger, M.J., et al., *Impact of influenza on mortality in relation to age and underlying disease, 1967-1989*. Int J Epidemiol, 1993. 22(2): p. 334-40.
3. van Essen, G.A., et al., *Influenza vaccination in 2000: recommendations and vaccine use in 50 developed and rapidly developing countries*. Vaccine, 2003. 21(16): p. 1780-5.
4. *Committee for Proprietary Medicinal Products (CHMP). Note for Guidance on Harmonisation of Requirements for Influenza Vaccines*. (CHMP/BWP/214/96), (adopted March 1997).
5. Beyer, W.E., et al., *Seroprotection rate, mean fold increase, seroconversion rate: which parameter adequately expresses seroresponse to influenza vaccination?* Virus Res, 2004. 103(1-2): p. 125-32.
6. McElhaney, J.E., et al., *The effect of influenza vaccination on IL2 production in healthy elderly: implications for current vaccination practices*. J Gerontol, 1992. 47(1): p. M3-8.
7. McElhaney, J.E., et al., *Vaccine-related determinants of the interleukin-2 response to influenza vaccination in healthy young and elderly adults*. Vaccine, 1995. 13(1): p. 6-10.
8. Powers, D.C. and R.B. Belshe, *Effect of age on cytotoxic T lymphocyte memory as well as serum and local antibody responses elicited by inactivated influenza virus vaccine*. J Infect Dis, 1993. 167(3): p. 584-92.
9. Provinciali, M., et al., *Impaired antibody response to influenza vaccine in institutionalized elderly*. Ann N Y Acad Sci, 1994. 717: p. 307-14.
10. Kang, I., et al., *Age-associated change in the frequency of memory CD4+ T cells impairs long term CD4+ T cell responses to influenza vaccine*. J.Immunol., 2004. 173: p. 673-81.
11. Remarque, E.J., et al., *Correlation between the antibody response to influenza vaccine and helper T cell subsets in healthy aging*. Vaccine, 1996. 14(2): p. 127-30.
12. Deng, Y., et al., *Age-related impaired type 1 T cell responses to influenza: reduced activation ex vivo, decreased expansion in CTL culture in vitro, and blunted response to influenza vaccination in vivo in the elderly*. J Immunol, 2004. 172(6): p. 3437-46.
13. Kohut, M.L., et al., *Moderate exercise improves antibody response to influenza immunization in older adults*. Vaccine, 2004. 22(17-18): p. 2298-306.
14. Kubiet, M.A., et al., *Serum antibody response to influenza vaccine in pulmonary patients receiving corticosteroids*. Chest, 1996. 110(2): p. 367-70.
15. Ebell, M.H., L.L. White, and T. Casault, *A systematic review of the history and physical examination to diagnose influenza*. J Am Board Fam Pract, 2004. 17(1): p. 1-5.
16. Walsh, E.E., C. Cox, and A.R. Falsey, *Clinical features of influenza A virus infection in older hospitalized persons*. J Am Geriatr Soc, 2002. 50(9): p. 1498-503.
17. Zambon, M., et al., *Diagnosis of influenza in the community: relationship of clinical diagnosis to confirmed virological, serologic, or molecular detection of influenza*. Arch Intern Med, 2001. 161(17): p. 2116-22.
18. Connolly, A.M., et al., *What are the complications of influenza and can they be prevented? Experience from the 1989 epidemic of H3N2 influenza A in general practice*. BMJ, 1993. 306(6890): p. 1452-4.
19. McBean, A.M., J.D. Babish, and J.L. Warren, *The impact and cost of influenza in the elderly*. Arch Intern Med, 1993. 153(18): p. 2105-11.
20. Foster, D.A., et al., *Influenza vaccine effectiveness in preventing hospitalization for pneumonia in the elderly*. Am J Epidemiol, 1992. 136(3): p. 296-307.
21. Nichol, K.L., *Complications of influenza and benefits of vaccination*. Vaccine, 1999. 17 Suppl 1: p. S47-52.

22. Nichol, K.L., et al., *The effectiveness of vaccination against influenza in healthy, working adults*. N Engl J Med, 1995. 333(14): p. 889-93.
23. Armstrong, B., et al., *Effect of influenza vaccination on excess death occurring during periods of high circulation of influenza: cohort study in elderly people*. BMJ, 2004. doi:10.1136/BMJ.38198.594109.AE((published 15 August 2004)).
24. Voordouw, A.C.G., et al., *Annual revaccination against influenza and mortality risk in community dwelling elderly persons*. JAMA, 2004. 292(17): p. 2089-95.
25. Voordouw, B.C., et al., *Influenza vaccination in community-dwelling elderly: impact on mortality and influenza-associated morbidity*. Arch Intern Med, 2003. 163(9): p. 1089-94.
26. Mullooly, J.P., et al., *Influenza vaccination programs for elderly persons: cost-effectiveness in a health maintenance organization*. Ann Intern Med, 1994. 121(12): p. 947-52.
27. Wang, S., et al., *Economic evaluation of vaccination against influenza in the elderly: n experience from a population-based influenza vaccination program in Taiwan*. Vaccine, 2005. 23: p. 1973-80.
28. Puig-Barbera, J., et al., *Reduction in hospital admissions for pneumonia in non-institutionalised elderly people as a result of influenza vaccination: a case-control study in Spain*. J Epidemiol Community Health, 1997. 51(5): p. 526-30.
29. Nichol, K.L. and M. Goodman, *Cost effectiveness of influenza vaccination for healthy persons between ages 65 and 74 years*. Vaccine, 2002. 20 Suppl 2: p. S21-4.
30. Nichol, K.L., et al., *The efficacy and cost effectiveness of vaccination against influenza among elderly persons living in the community*. N Engl J Med, 1994. 331(12): p. 778-84.
31. Ahmed, A.E., K.G. Nicholson, and J.S. Nguyen-Van-Tam, *Reduction in mortality associated with influenza vaccine during 1989-90 epidemic*. Lancet, 1995. 346(8975): p. 591-5.
32. Landi, F., et al., *Effects of influenza vaccination on mortality among frail, community-living elderly patients: an observational study*. Aging Clin Exp Res, 2003. 15(3): p. 254-8.
33. *From the Centers for Disease Control and Prevention. Pneumonia and influenza death rates – United States, 1979-1994*. JAMA, 1995. 274(7): p. 532.
34. Nieuwsbrief influenza surveillance 2004/2005, 2005. Jaargang 13(nr.9): p. (http://www.virology.nl).
35. Govaert, T.M., et al., *The efficacy of influenza vaccination in elderly individuals. A randomized double-blind placebo-controlled trial*. JAMA, 1994. 272(21): p. 1661-5.
36. Hak, E., et al., *Influence of high-risk medical conditions on the effectiveness of influenza vaccination among elderly members of 3 large managed-care organizations*. Clin Infect Dis, 2002. 35(4): p. 370-7.
37. Nichol, K.L., et al., *Influenza vaccination and reduction in hospitalizations for cardiac disease and stroke among the elderly*. N Engl J Med, 2003. 348(14): p. 1322-32.
38. Beyer, W.E., et al., *Effects of repeated annual influenza vaccination on vaccine sero- response in young and elderly adults*. Vaccine, 1996. 14(14): p. 1331-9.
39. de Bruijn, I.A., et al., *Annually repeated influenza vaccination improves humoral responses to several influenza virus strains in healthy elderly*. Vaccine, 1997. 15(12-13): p. 1323-9.
40. Beyer, W.E., et al., *Protection against influenza after annually repeated vaccination: a meta-analysis of serologic and field studies*. Arch Intern Med, 1999. 159(2): p. 182-8.
41. Dindinaud, G., et al., *Influenza epidemic among a community of elderly people in spite of vaccination*. Eur J Epidemiol, 1993. 9(6): p. 667-70.
42. Saah, A.J., et al., *Influenza vaccine and pneumonia mortality in a nursing home population*. Arch Intern Med, 1986. 146(12): p. 2353-7.
43. Patriarca, P.A., et al., *Efficacy of influenza vaccine in nursing homes. Reduction in illness and complications during an influenza A (H3N2) epidemic*. JAMA, 1985. 253(8): p. 1136-9.
44. Arden, N., A.S. Monto, and S.E. Ohmit, *Vaccine use and the risk of outbreaks in a sample of nursing homes during an influenza epidemic*. Am J Public Health, 1995. 85(3): p. 399-401.

45. Nicholson, K.G., et al., *Acute upper respiratory tract viral illness and influenza immunization in homes for the elderly*. Epidemiol Infect, 1990. 105(3): p. 609-18.
46. Barker, W.H. and J.P. Mullooly, *Pneumonia and influenza deaths during epidemics: implications for prevention*. Arch Intern Med, 1982. 142(1): p. 85-9.
47. Keitel, W.A., T.R. Cate, and R.B. Couch, *Efficacy of sequential annual vaccination with inactivated influenza virus vaccine*. Am J Epidemiol, 1988. 127(2): p. 353-64.
48. Keitel, W.A., et al., *Efficacy of repeated annual immunization with inactivated influenza virus vaccines over a five year period*. Vaccine, 1997. 15(10): p. 1114-22.
49. Goronzy, J.J., et al., *Value of immunological markers in predicting responsiveness to influenza vaccination in elderly individuals*. J Virol, 2001. 75(24): p. 12182-7.
50. Clancy, R.L., *Mucosal vaccines for the prevention of influenza*. Drugs, 1995. 50(4): p. 587-94.
51. McElhaney, J.E., et al., *Split-virus influenza vaccines: do they provide adequate immunity in the elderly?* J Gerontol, 1994. 49(2): p. M37-43.
52. Glathe, H., S. Bigl, and A. Grosche, *Comparison of humoral immune responses to trivalent influenza split vaccine in young, middle-aged and elderly people*. Vaccine, 1993. 11(7): p. 702-5.
53. Huang, Y.P., et al., *The relationship between influenza vaccine-induced specific antibody responses and vaccine-induced nonspecific autoantibody responses in healthy older women*. J Gerontol, 1992. 47(2): p. M50-5.
54. McElhaney, J.E., et al., *Antibody response to whole-virus and split-virus influenza vaccines in successful ageing*. Vaccine, 1993. 11(10): p. 1055-60.
55. McElhaney, J.E., et al., *Granzyme B: a marker of risk for influenza in institutionalized older adults*. Vaccine, 2001. 19(27): p. 3744-51.
56. Powers, D.C., *Effect of age on serum immunoglobulin G subclass antibody responses to inactivated influenza virus vaccine*. J Med Virol, 1994. 43(1): p. 57-61.
57. Meyers, D.G., *Could influenza vaccination prevent myocardial infarction, stroke and sudden cardiac death?* Am J Cardiovasc Drugs, 2003. 3(4): p. 241-4.
58. Naghavi, M., et al., *Influenza infection exerts prominent inflammatory and thrombotic effects on the atherosclerotic plaques of apolipoprotein E-deficient mice*. Circulation, 2003. 107(5): p. 762-8.
59. Stewart, S. and e. al., *Heart failure in a cold climate. Seasonal variation in heart failure-related morbidity and mortality,*. J Am Coll Cardiol, 2002: p. 760-6.
60. Madjid, M., et al., *Influenza and cardiovascular disease: a new opportunity for prevention and the need for further studies*. Circulation, 2003. 108(22): p. 2730-6.
61. Engblom, E., et al., *Fatal influenza A myocarditis with isolation of virus from the myocardium*. Acta Med Scand, 1983. 213(1): p. 75-8.
62. Drescher, J., et al., *Recent influenza virus A infections in forensic cases of sudden unexplained death*. Arch Virol, 1987. 92(1-2): p. 63-76.
63. Smeeth, L., et al., *Risk of myocardial infarction and stroke after acute infection or vaccination*. N Engl J Med, 2004. 351(25): p. 2611-8.
64. Falsey, A.R., et al., *Acute respiratory tract infection in daycare centers for older persons*. J Am Geriatr Soc, 1995. 43(1): p. 30-6.
65. Glathe, H. and W. Lange, *Influenza vaccination in older patients. Immunogenicity, epidemiology and available agents*. Drugs Aging, 1995. 6(5): p. 368-87.
66. Nicholson, K.G., et al., *Acute viral infections of upper respiratory tract in elderly people living in the community: comparative, prospective, population based study of disease burden*. BMJ, 1997. 315(7115): p. 1060-4.
67. Nicholson, K.G., *Human Influenza*, in *Textbook of influenza*, K.G. Nicholson, R.G. Webster, and H. A.J., Editors. 1998, reprint 2000, Blackwell sciences ltd.: London. p. 233-244.

68. Howells, C.H., et al., *Influenza vaccination and mortality from bronchopneumonia in the elderly*. Lancet, 1975. 1(7903): p. 381-3.
69. Gross, P.A., et al., *The efficacy of influenza vaccine in elderly persons. A meta-analysis and review of the literature*. Ann Intern Med, 1995. 123(7): p. 518-27.
70. Barker, W.H. and J.P. Mullooly, *Influenza vaccination of elderly persons. Reduction in pneumonia and influenza hospitalizations and deaths*. JAMA, 1980. 244(22): p. 2547-9.
71. Nichol, K.L., J. Wuorenma, and T. von Sternberg, *Benefits of influenza vaccination for low-, intermediate-, and high- risk senior citizens*. Arch Intern Med, 1998. 158(16): p. 1769-76.
72. de Jong, J.C., et al., *Haemagglutination-inhibiting antibody to influenza virus*. Dev Biol (Basel), 2003. 115: p. 63-73.
73. Pyrhonen, S., J. Suni, and M. Romo, *Clinical trial of a subunit influenza vaccine*. Scand J Infect Dis, 1981. 13(2): p. 95-9.
74. Smith, D.J., et al., *Variable efficacy of repeated annual influenza vaccination*. Proc Natl Acad Sci U S A, 1999. 96(24): p. 14001-6.
75. Smith, D.J., et al., *Mapping of the antigenic and genetic evolution of influenza virus*. Science, 2004. 305: p. 371-376.
76. Ben-Yehuda, A., et al., *The influence of sequential annual vaccination and of DHEA administration on the efficacy of the immune response to influenza vaccine in the elderly*. Mech Ageing Dev, 1998. 102(2-3): p. 299-306.
77. Cassetti, M., et al., *Report of meeting on the development of influenza vaccines with broad spectrum and long-lasting immune responses, World Health Organization, Geneva, Switzerland, 26-27 February 2004*. Vaccine, 2005. 23(13): p. 1529-1533.
78. de Jong, J.C., et al., *Mismatch between the 1997/1998 influenza vaccine and the major epidemic A(H3N2) virus strain as the cause of an inadequate vaccine-induced antibody response to this strain in the elderly*. J Med Virol, 2000. 61(1): p. 94-9.
79. Govaert, T.M., et al., *Immune response to influenza vaccination of elderly people. A randomized double-blind placebo-controlled trial*. Vaccine, 1994. 12(13): p. 1185-9.
80. Wongsurakiat, P., et al., *Acute Respiratory Illness in Patients With COPD and the Effectiveness of Influenza Vaccination: A Randomized Controlled Study*. Chest, 2004. 125(6): p. 2011-2020.
81. Pawelec, G., et al., *Is human immunosenescence clinically relevant? Looking for "immunological risk phenotypes."* Trends Immunol., 2002. 23(7): p. 330-2.
82. Nordin, J., et al., *Influenza vaccine effectiveness in preventing hospitalizations and deaths in persons 65 years or older in Minnesota, New York, and Oregon: data from 3 health plans*. J Infect Dis, 2001. 184(6): p. 665-70.
83. Nichol, K.L., et al., *Effectiveness of influenza vaccine in the elderly*. Gerontology, 1996. 42(5): p. 274-9.
84. Munoz, E. and M. Deem, *Epitope analysis for influenza vaccine design*. Vaccine, 2005. 23: p. 1144-8.
85. Hak, E., et al., *Confounding by indication in non-experimental evaluation of vaccine effectiveness: the example of prevention of influenza complications*. J Epidemiol Community Health, 2002. 56(12): p. 951-5.
86. Hak, E., et al., *Development and validation of a clinical prediction rule for hospitalization due to pneumonia or influenza or death during influenza epidemics among community-dwelling elderly persons*. J Infect Dis, 2004. 189(3): p. 450-8.
87. Nichol, K.L. and P. Mendelman, *Influence of clinical case definitions with differing levels of sensitivity and specificity on estimates of the relative and absolute health benefits of influenza vaccination among healthy working adults and implications for economic analyses*. Virus Res, 2004. 103(1-2): p. 3-8.

88. Hennekes, C.H. and J.E. Buring, *Cohort studies*, in *Epidemiology in medicine*, S.L. Mayrent, Editor. 1987, Lippincott Williams & Wilkins: Philadelphia. p. 169.
89. Widdicombe, J. and K. S., *Acute cough in the elderly: aetiology, diagnosis and therapy*. Drugs Aging, 2004. 21(4): p. 243-58.
90. Hennekes, C.H. and J.E. Buring, *Basic principles - the role of confounding*, in *Epidemiology in medicine*, S.L. Mayrent, Editor. 1987, Lippincott Williams & Wilkins: Philadelphia. p. 35-7.
91. Vlug, A., et al., *Postmarketing surveillance based on electronic patient records: The IPCI project*. Meth Inform Med, 1999. 38: p. 339-44.
92. Nicholson, K.G., *History of inactivated influenza vaccines*, in *Textbook of influenza*, K.G. Nicholson, R.G. Webster, and H. A.J., Editors. 1998, reprint 2000, Blackwell sciences ltd.: London. p. 317-23.

9

Summary & Samenvatting

1 Summary

Since the last 400 years, recurrent epidemics and incidental pandemics caused by influenza viruses are documented. However, an influenza virus was not identified until 1933 and the first influenza vaccine was licensed in 1945 [1]. Influenza viruses circulate throughout the year, however in the northern hemisphere, influenza epidemic activity is mainly confined to winter months. In the Netherlands, an influenza epidemic is declared when at least 15 per 10,000 inhabitants report symptoms of influenza like illness (i.e., the epidemic threshold) [2].

Especially in the elderly and in persons with chronic underlying diseases, influenza infections are associated with morbidity and mortality. In the Netherlands, the influenza epidemics have been of mild to moderate severity in the last decade.

Annual vaccination programs aim to reduce the burden of disease associated with influenza infections, especially in elderly and in those with chronic underlying disease. However, despite recommendations to annually vaccinate all those who fulfil the inclusion criteria for participation in these programs, little is known about the effectiveness of influenza vaccines when administered annually.

The first aim of this thesis was to get better insight into this effectiveness of influenza vaccination in community dwelling elderly who were immunised as part of the national influenza vaccination program. To investigate this, 4 observational studies were conducted which are described in this thesis (chapters 2-5).

Since strains of influenza viruses may change, the composition of influenza vaccines is reassessed each year. The composition is based on recommendation of the World Health Organisation. Influenza vaccines need to be licensed for a first time and when the antigenic composition changes the vaccine needs to be relicensed. For this procedure the regulatory authorities within the European Union request an immunogenicity trial in a predefined number of healthy adults and elderly. A second aim of this thesis was to describe the outcomes of these serological trials and to investigate the influence of population characteristics on these outcomes (chapter 6).

Immunogenicity data are called “surrogate” parameters, which may be used as a proxy for clinical efficacy (i.e., the correlate for protection), provided that it is validated against the relevant clinical outcome. Since influenza vaccines were first licensed 60 years ago, clinical trials addressing both serological and clinical outcomes are generally not performed. Immunogenicity data are usually gathered in serological trials, whereas clinical outcomes are mostly investigated in observational studies.

A final aim of this thesis was to investigate the impact of influenza vaccination on immunological and clinical outcomes, and the substantiation of using serological parameters as a correlate for clinical protection. For this purpose, we performed a review which is described in chapter 7.

Chapter 1 describes the background of this thesis. In this chapter a short historic overview is given, followed by a summary of the epidemiology of influenza infections, symptomatology of influenza, the goals of influenza vaccination and how the vaccination program in the Netherlands is designed. Furthermore, this chapter shortly summarises what is known from the literature with respect to the immune response and clinical protection to influenza vaccines, especially in elderly. And finally the criteria for annual relicensure of influenza vaccines are shortly discussed.

Chapter 2 describes the first cohort study we performed in the Integrated Primary Care Information (IPCI) database with the objective to investigate the effectiveness of influenza vaccination in community dwelling elderly. For this study, we selected an eligible elderly population during the 1996-1997 influenza epidemic. Eligibility was defined as an age of 65 years or older on January 1st of 1996 and a permanent status in a practice in the IPCI source population. Two cohorts were defined on the basis of vaccination status. We estimated and compared all cause mortality, pneumonia and clinical influenza infection risk between the cohorts. This study was conducted in a season of mild-moderate influenza activity and good antigenic match between vaccine strains and circulating strains and influenza vaccination was associated with decreased mortality and influenza infections in community dwelling elderly.

Chapter 3 focused on revaccination since most large scale cohort studying effectiveness of influenza vaccination did not systematically address the clinical benefit of annual revaccinations. The aim of the study was to investigate the effect of annual influenza revaccination on mortality in community dwelling elderly. In this population-based cohort study the eligible elderly population was selected during the period 1996 through 2002. For each year, the individual cumulative exposure to influenza vaccination since study entry was computed. Eligibility was defined as an age of 65 years or older on January 1st of the year of study entry. In this study, the association between the number of consecutive influenza vaccinations and all cause mortality was compared to no vaccination using a time-varying multivariate Cox-proportional hazard model, adjusted for age, gender, chronic respiratory and cardiovascular disease, hypertension, diabetes mellitus, renal failure, and cancer.

The study population included 26,071 subjects of whom 3,485 died during follow-up. Overall, a first vaccination was associated with a non-significant annual reduction of mortality risk of 10% ($CI_{95\%}$: -3%- 22%), revaccination reduced mortality risk by 24% ($CI_{95\%}$: 17-30%). Compared to a first vaccination, revaccination was associated with a reduced annual mortality risk of 15% ($CI_{95\%}$: 4-25%). During the epidemic periods this reduction was 28% ($CI_{95\%}$: 4-47%). Similar estimates were obtained for subjects with and without chronic comorbidity and those aged 70 years or older at baseline. Overall, influenza vaccination prevented one death for every 302 vaccinees at a vaccination coverage that varied between 64% and 74%. This was the first study

in which we showed that annual influenza vaccination was associated with a reduction in all cause mortality risk in a population of community dwelling elderly, also in those at higher age.

In chapter 4 we further explored the possible benefit of annual revaccination on the risk of sudden cardiac death. Influenza infections are associated with an increased risk of acute cardiac events. Although a protective effect of influenza vaccination on the risk of sudden cardiac death (SCD) has been suggested, it has not been systematically studied. The cohort study was performed in the same study population as described in chapter 3, with the exclusion of those persons in whom a malignancy was diagnosed. In this study, the risk of SCD after a first vaccination or a revaccination was compared to no vaccination using a time-varying multivariate Cox-proportional hazard model, adjusted for age, gender, smoking and underlying chronic disease. Overall, any influenza vaccination or revaccination was associated with a non-significant risk reduction of SCD. An increased risk following a first vaccination was observed, which reached statistical significance in subjects without comorbidity and in those with baseline age<70 years. Despite modest results, the study supports the practice of annual vaccination The observed increased risk following a first vaccination needs further attention.

Chapter 5 describes a cohort study in which the effect of annual influenza vaccination on the occurrence of lower respiratory tract infections, defined as acute bronchitis, acute exacerbation of chronic bronchitis and pneumonia (LRTI) in community dwelling elderly was investigated. Acute bronchitis is the most common complication following influenza infection and the risk increases in elderly and in those with underlying chronic conditions. However, little is known about the effectiveness of influenza vaccination in reducing the risk, and no studies were performed addressing the effect of revaccination on LRTI. In our study, a first vaccination was not associated with a reduced LRTI risk. During epidemic periods, revaccination reduced LRTI risk by 33% ($CI_{95\%}$: 8-52%), in individuals without comorbidity.

Chapter 6 describes serological data from a large number of trials that were submitted to the Dutch Medicines Evaluation Board between 1992 and 2002, as part of an annual relicensure requirement. The trials were analysed according to the requirements and criteria described in the so-called Committee of Human Medicinal Products Note for Guidance on harmonisation of requirements for influenza vaccines (CHMP NfG). This guidance document is meant to assist marketing authorisation holders in the planning, conduct and evaluation of the preclinical and clinical studies for their regulatory annual relicensure dossier. Of the 48 age-defined trials, all but three trials fulfilled the CHMP criteria. Increasing pre-vaccination antibody levels significantly contributed to the fulfilment of the seroprotection criterion and post-vaccination parameters were strongly dependent on age. Trials with “younger”

vaccinees appeared to have better chances to meet all criteria. History of previous influenza vaccinations significantly affected pre-, but not post-vaccination levels. The CHMP criteria showed serious methodological limitations, which affected their ability to answer their objective, i.e., determination of the immunogenic potential of the individual trivalent inactivated influenza vaccine. The value and need of these trials for annual relicensure purposes may be questioned.

Chapter 7 is a review to investigate the impact of annual revaccination on serological and clinical outcomes, and the validity of serological parameters as a proxy for clinical protection. Efficacy of annual variations in influenza vaccines is measured by serological parameters, and little is known about their correlation with clinical protection, especially when administered annually and particularly in elderly individuals. Following an ongoing Medline search, publications of studies based upon use of inactivated trivalent influenza vaccines in elderly subjects and relevant reviews were selected. In 23 serology publications no correlations were found between postvaccination seroprotection rate and seroresponse. In 30 publications addressing clinical outcomes a significant protective effect with regard to death, pneumonia or influenza was found. This review supports the clinical benefit of annual influenza vaccination in elderly, but does not support the use of serological data as proxy for clinical outcomes. The value of serological endpoints as correlate for clinical protection remains undetermined also due to a surprising lack of studies correlating these outcomes. The main question that remains is why clinical protection sustains with compliance to annual revaccination when seroresponsiveness decreases with increasing age and following repeated vaccination.

Chapter 8 is the general discussion of the thesis. In this chapter, the main findings of different studies are summarised and put in the context of other published research. This chapter also discussed some of the methodological issues. This chapter concludes with some ideas for further research and regulatory guidance for annual relicensure of influenza vaccines.

References

1. Nicholson, K.G., *Historical introduction*, in *Textbook of influenza*, K.G. Nicholson, R.G. Webster, and H. A.J., Editors. 1998, reprint 2000, Blackwell sciences ltd.: London. p. 3-26.
2. NIVEL, *Nederlands Instituut voor de eerste lijns gezondheidszorg. Continue morbiditeitsregistratie peilstations Nederland 1988/89*. 1991, Nivel: Utrecht.

2 Samenvatting

Gedurende de laatste 400 jaar zijn influenza epidemieën regelmatig en pandemieën incidenteel gedocumenteerd. Het influenza virus werd echter pas voor het eerst in 1933 geïdentificeerd en het eerste influenza vaccin kwam in 1945 beschikbaar [1]. Het hele jaar is er circulatie van influenza virussen, maar op het noordelijk halfrond zien we influenza epidemieën eigenlijk alleen in de wintermaanden. In Nederland wordt een influenza epidemie vastgesteld wanneer tenminste 15 van de 10.000 inwoners symptomen van een influenza-achtig ziektebeeld hebben. Dit noemen we de epidemische drempel [2].

Vooral ouderen en personen met chronisch onderliggend lijden hebben een verhoogd risico op complicaties of overlijden ten gevolge van influenza infecties. De laatste 10 jaar hebben we in Nederland geen grote influenza epidemieën meer gehad.

Het doel van de jaarlijkse influenza vaccinatie campagne is om ziektelast ten gevolge van influenza infecties te verminderen, vooral bij oudere personen en personen met chronische ziekten. Er is echter weinig bekend over de effectiviteit van het jaarlijks vaccineren tegen influenza.

Het eerste doel van het onderzoek beschreven in dit proefschrift was om een beter inzicht te krijgen in de effectiviteit van influenza vaccinatie bij zelfstandig wonende oudere personen die jaarlijks worden gevaccineerd in het kader van het nationaal influenza vaccinatie programma. In totaal werden 4 observationele studies uitgevoerd. Deze zijn beschreven in de hoofdstukken 2-5.

Omdat de circulerende influenzavirussen kunnen veranderen, wordt de samenstelling van influenzavaccins jaarlijks opnieuw beoordeeld door de Wereld Gezondheid Organisatie. Een nieuw influenzavaccin wordt eenmaal volledig beoordeeld bij een eerste registratie, waarna jaarlijks uitsluitend de veranderde samenstelling wordt beoordeeld en opnieuw geregistreerd. De Europese registratie autoriteiten vereisen voor deze jaarlijkse herregistratie een zogenaamde immunogeniciteitstudie bij tenminste 50 gezonde volwassen en tenminste 50 gezonde oudere personen. Het tweede doel van het onderzoek was om de resultaten van deze serologie studies te beschrijven en om de mogelijke invloed van populatie factoren op de uitkomsten te onderzoeken (hoofdstuk 6).

Immunogeniciteit of serologische parameters worden als “surrogaat” parameters beschouwd voor klinische werkzaamheid, op voorwaarde dat deze gevalideerd zijn voor een relevante klinische uitkomst. Influenza vaccins zijn echter 60 jaar geleden voor het eerst geregistreerd. Daarom zijn er nauwelijks experimentele klinische studies waarbij serologische en klinische uitkomsten beiden zijn onderzocht. De data

met betrekking tot immunogeniciteit komen in de regel uit serologie studies en de klinische uitkomsten uit observationeel onderzoek.

Het laatste doel van het huidige onderzoek was dan ook om de invloed van influenza vaccinatie op zowel de immunologische als klinische uitkomsten te onderzoeken in een poging om de validiteit van gebruikte serologische parameters voor klinische bescherming te onderbouwen. De uitkomst hiervan is beschreven in het overzichtsartikel in hoofdstuk 7.

Hoofdstuk 1 beschrijft de achtergrond van het onderzoek. Dit hoofdstuk geeft een kort historisch overzicht, een samenvatting van de epidemiologie van influenza infecties, de symptomatologie van influenza infecties, de doelen van influenzavaccinatie en legt uit hoe het vaccinatieprogramma in Nederland is ontworpen. Verder geeft dit hoofdstuk een korte samenvatting van de literatuur met betrekking tot de immuunrespons en de klinisch bescherming na influenza vaccinatie, vooral in oudere personen. Tenslotte worden de criteria voor het jaarlijks herregistreren van influenza vaccines kort bediscussieerd.

Hoofdstuk 2 beschrijft de eerste cohortstudie die we uitvoerden in de IPCI (Integrated Primary Care Information) database met als doel om de effectiviteit van influenza vaccinatie in ouderen te onderzoeken. Voor deze studie werd de influenza epidemie van 1996-1997 onderzocht in personen die op 1 januari 1996 vijfenzestig jaar of ouder waren, en die een permanente status in een deelnemende praktijk in de IPCI bronpopulatie hadden. Er werden 2 groepen gedefinieerd op basis van vaccinatie status. In deze groepen werd het verschil in risico op overlijden, het ontwikkelen van pneumonie of een klinische influenza infectie onderzocht. De influenza epidemie van 1996-1997 werd gekarakteriseerd door een milde tot matig ernstige influenza activiteit en een goede antigene “match” tussen circulerende influenza virusstammen en vaccinstammen. Vaccinatie was geassocieerd met een verminderde mortaliteit en minder influenza infecties bij zelfstandig wonende oudere personen.

In hoofdstuk 3 beschrijft het onderzoek de jaarlijkse influenzavaccinatie. Hoewel veel studies het effect van influenzavaccinatie hebben bestudeerd, is het klinisch nut van het jaarlijks vaccineren niet eerder systematisch onderzocht. Het doel van het onderzoek was daarom om het effect van jaarlijkse influenzavaccinatie op het risico op overlijden te onderzoeken bij oudere personen. Deze op de Nederlandse populatie gebaseerde cohortstudie liep van 1996-2002. Elk jaar werd de individuele cumulatieve vaccinatiestatus bepaald in de geselecteerde oudere personen. Personen werden geselecteerd indien ze 65 jaar of ouder waren op 1 januari van het jaar waarin zij in de studie startten (tussen 1996-2001). De associatie tussen het aantal opeenvolgende influenzavaccinaties en mortaliteit werd bestudeerd met personen die niet eerder waren gevaccineerd tegen influenza als vergelijkingsgroep. Hiervoor werd een in tijd variërend multivariate Cox-proportional hazard model ontwikkeld,

gecorrigeerd voor leeftijd, geslacht, chronische ziekten van luchtwegen, hart en bloedvaten, hypertensie, diabetes mellitus, chronische nierinsufficiëntie en kanker.

Van de 26071 geselecteerde personen overleden er 3485 gedurende de studie. In het algemeen leek een eerste vaccinatie geassocieerd met een niet-significante daling van 10% in het risico op overlijden gedurende het jaar (95% betrouwbaarheidsinterval ($BI_{95\%}$): -3%- 22%), revaccinatie verminderde echter het risico op overlijden met 24% ($BI_{95\%}$: 17-30%). Vergeleken met een 1ste vaccinatie was revaccinatie geassocieerd met een vermindering van het jaarlijkse overlijdensrisico van 15% ($BI_{95\%}$: 4-25%). Tijdens de influenza-epidemieën was deze vermindering zelfs 28% ($BI_{95\%}$: 4-47%). Vergelijkbare resultaten werden gevonden voor personen met of zonder onderliggend lijden en personen van 70 jaar of ouder aan het begin van de studie. In deze studie werd bij 304 gevaccineerden één overlijden voorkómen bij een vaccinatiegraad die varieerde tussen 64% en 74%. In deze eerste studie toonden we aan dat jaarlijkse influenza vaccinatie geassocieerd was met een vermindering in mortaliteit bij zelfstandig wonende oudere personen, ook op hogere leeftijd.

In hoofdstuk 4 wordt het effect van jaarlijkse vaccinatie op het risico van plotselinge hartdood beschreven. Influenza infecties zijn geassocieerd met een verhoogd risico op acute cardiale gebeurtenissen. Hoewel eerder was gesuggereerd dat influenzavaccinatie het risico op acute hartdood kon verminderen, was dit nog niet eerder systematisch onderzocht. Deze cohortstudie werd uitgevoerd in dezelfde populatie als omschreven in hoofdstuk 3, met uitzondering van personen waarin een maligniteit was gediagnosticeerd. Ook in deze studie werd het risico op acute hartdood na een eerste vaccinatie of een revaccinatie vergeleken met het risico in personen zonder vaccinatie, gebruik makend van een in tijd variërend multivariaat Cox-proportional hazard model, en gecorrigeerd voor leeftijd, geslacht, en onderliggende ziekten. In het algemeen leek influenzavaccinatie of revaccinatie te zijn geassocieerd met een niet-significante vermindering van het risico op acute hartdood. Er was een verhoogd risico na een eerste vaccinatie, dat statistisch significant bleek in de groep personen zonder onderliggend lijden en in diegenen die jonger waren dan 70 jaar aan het begin van de studie. Deze resultaten vormen een ondersteuning voor het belang van jaarlijkse vaccinatie.

Hoofdstuk 5 beschrijft een cohortstudie waarin het effect werd onderzocht van jaarlijkse vaccinatie op het risico op lagere luchtweginfecties (acute bronchitis, acute exacerbatie van chronische bronchitis en pneumonie) bij zelfstandig wonende ouderen. Acute bronchitis is de meest voorkomende complicatie na een influenza infectie en het risico neemt toe bij hogere leeftijd en bij onderliggende chronische ziekten. Maar er is weinig bekend over de effectiviteit van influenza vaccinatie, vooral in relatie tot acute bronchitis, en er zijn geen studies die de relatie tussen het risico op lagere luchtweginfectie en revaccinatie hebben onderzocht.

In onze studie was een eerste vaccinatie niet geassocieerd met een verminderd risico op lagere luchtweginfecties. Tijdens de influenza epidemieën verminderde het risico na revaccinatie echter met 33% ($BI_{95\%}$: 8-52%) bij personen zonder onderliggende chronische ziekten.

Hoofdstuk 6 beschrijft de serologische resultaten van een groot aantal studies, die gedurende de periode 1992-2002 bij het College ter Beoordeling van Geneesmiddelen werden ingediend als onderdeel van de jaarlijkse verplichting van de registratiehouders om de nieuwe samenstelling van hun influenza vaccin te laten herregistreren.

De studies werden geanalyseerd volgens de vereisten zoals deze zijn beschreven in de zogenaamde Committee of Human Medicinal Products Note for Guidance on harmonisation of requirements for influenza vaccines (CHMP NfG). Deze richtlijn is bedoeld om registratiehouders bij te staan in de planning, uitvoering en evaluatie van de preklinische en klinische studies voor de jaarlijkse herregistratie. Van de 48 studies voldeden er 45 aan de CHMP criteria. Hogere prevaccinatie antistofconcentraties droegen significant bij aan het postvaccinatie seroprotectie criterium. De postvaccinatie parameters waren sterk afhankelijk van leeftijd. Studies waarin de gevaccineerden een gemiddeld lagere leeftijd hebben, voldeden vaker aan alle criteria. Een voorgeschiedenis van influenzavaccinatie had een statistisch significante invloed op de antistofconcentraties voorafgaand aan vaccinatie maar niet op de antistofconcentraties na vaccinatie. De CHMP criteria bleken ernstige methodologische beperkingen te hebben, die van invloed waren op hun mogelijkheid om de immunogeniciteit van de individuele geïnactiveerde trivalente influenzavaccins vast te stellen. De waarde en noodzaak van deze studies voor de jaarlijkse herregistratie van de geïnactiveerde influenza vaccins zou ter discussie gesteld moeten worden.

Hoofdstuk 7 is een overzichtsartikel over de invloed van het jaarlijks vaccineren tegen influenza op serologische en klinische uitkomsten en de validiteit van de gebruikte serologische parameters voor klinische bescherming. De effectiviteit van de jaarlijkse variaties in influenzavaccins wordt gemeten aan de hand van serologische parameters en er is weinig bekend over hun correlatie met klinische bescherming, vooral bij het jaarlijks vaccineren en bij oudere personen.

Via Medline werden publicaties geselecteerd van studies, die het gebruik van geïnactiveerde trivalente influenza vaccins bij oudere personen beschreven, alsmede relevante overzichtsartikelen. In 23 publicaties met serologische gegevens werden geen correlaties gevonden tussen de postvaccinatie seroprotectie graad en de serorespons. In 30 publicaties met klinische uitkomsten werd een significant beschermend effect van influenzavaccinatie gevonden voor de uitkomsten dood, pneumonie of influenza. Dit hoofdstuk bevestigt het klinisch nut van jaarlijkse vaccinatie van oudere personen. Het vormt echter geen ondersteuning voor het gebruik van sero-

logische parameters in plaats van klinische uitkomsten in studies. De waarde van serologische eindpunten als surrogaat parameter voor klinische bescherming blijft onduidelijk, mede door een opvallend tekort aan studies waarin beide uitkomsten zijn gemeten en gecorreleerd. De belangrijkste vraag blijft waarom klinische bescherming aanhoudt bij diegenen die zich jaarlijks laten vaccineren tegen influenza, terwijl de serorespons lijkt te verminderen bij het toenemen van de leeftijd en na herhaalde vaccinatie.

Hoofdstuk 8 is de algemene discussie. In dit hoofdstuk worden de belangrijkste bevindingen uit de verschillende studies samengevat en besproken in de context van de gepubliceerde literatuur. Ook bespreken we in dit hoofdstuk enkele methodologische punten.

Tenslotte eindigt het hoofdstuk met enkele ideeën voor verder onderzoek en de jaarlijkse herregistratie van influenza vaccins .

Referenties

1. Nicholson, K.G., *Historical introduction*, in *Textbook of influenza*, K.G. Nicholson, R.G. Webster, and H. A.J., Editors. 1998, reprint 2000, Blackwell sciences ltd.: London. p. 3-26.
2. NIVEL, *Nederlands Instituut voor de eerste lijns gezondheidszorg. Continue morbiditeitsregistratie peilstations Nederland 1988/89*. 1991, Nivel: Utrecht.

Dankwoord

Dan nu het leukste deel, het dankwoord. Voor iemand die gewend is altijd teveel woorden te gebruiken ook nog een hele kunst om dat enigszins binnen de perken te houden.

> Science is organized knowledge. Wisdom is organized life (Immanuel Kant)

Allereerst zijn er mijn promotoren Prof. dr. B.H.Ch. Stricker en Prof. Dr. J. van der Lei en mijn copromotor Dr. M.C.J.M. Sturkenboom.

Bruno, toen je na onze pilot studie zei, met of zonder mij, verder te willen met dit project, had ik niet gedacht dat ik me werkelijk nog eens voor een promotie onderzoek zou laten strikken. Maar het is je toch (mede door de overtuigingskracht van professor van der Noordaa) gelukt en ik heb er geen spijt van gehad. Het probleem van jaren vanuit één perspectief naar een onderwerp kijken is dat je denkt er veel van te weten. Je vragen, kritische opmerkingen en discussies hebben me laten zien dat er nog heel veel te leren was en is. Ik ben onder de indruk van de doortastendheid waarmee je dit project hebt getrokken, terwijl er toch aardig wat beren op de weg bleken. Vooral ook door je geloof in de goede afloop ("als je nu niet promoveert ligt het in ieder geval niet aan mij") en je aanstekelijk enthousiasme heb ik erg genoten van deze 4 jaar. Ik hoop ook echt dat we de samenwerking in de toekomst kunnen voortzetten.

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Miriam, je bent misschien wel eens moedeloos geworden van me. Maar geloof me, ik heb al je aanwijzingen, kritiek en ideeën steeds "aan boord genomen" en heb het erg gewaardeerd. Je methodologische kennis en vooral ook je schrijftalent heeft een grote indruk op me gemaakt. Verder weet ik nog steeds niet hoe je zoveel werk kan verzetten met 2 kleine kinderen (je commentaren waren bijna altijd als eerste binnen!!).

Heel erg bedankt leden van de leescommissie, voor de bereidheid het manuscript te beoordelen en zitting te nemen in de promotiecommissie.

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alles mee begonnen was. Jaren terug spraken we al over de klinische relevantie van de serologische data, nu staat het op papier.

Professor Osterhaus, vier jaar geleden, tijdens ons eerste gesprek, kwam er direct een stortvloed van ideeën over me heen. Deze waren helaas op dat moment niet direct uitvoerbaar, maar ze hebben wel geleid tot een productieve samenwerking met de afdeling Virologie. Bedankt hiervoor.

Professor Coutinho sprak ik jaren terug, tijdens de module infectieziekten epidemiologie in het kader van mijn MPH opleiding, over mijn plannen om influenza onderzoek te doen. Het werd uiteindelijk Rotterdam, maar gelukkig bent u er toch nog bij betrokken.

> We don't accomplish anything in this world alone ... and whatever happens is the result of the whole tapestry of one's life and all the weavings of individual threads from one to another that creates something (Sandra Day O'Connor).

Geen enkel stuk schrijf je alleen.

Paul van der Linden was mijn steun en toeverlaat bij de pilot studie. Als FoxPro-leek zat ik daar met twee linker handen. Bedankt voor alle hulp.

Zonder Jeanne had ik de datasets in FoxPro voor de revaccinatie studie nooit voor elkaar gekregen. Bovendien ben je een kei in het kritisch analyseren van data en manuscripten.

Derek Smith, you are scientifically one of the most original people I have met. Thanks for all the time you were willing to spent to elaborate on revaccination and effectiveness and the possible impact of (temporarily) interruption, but also all your efforts to improve the manuscripts and correct my English.

Professor Stijnen bedankt voor uw statistisch "vernuft". Leeftijd in dagen als follow-up en vaccinatie stoppers als aparte categorie, het waren gouden ideeën. Bovendien heel erg bedankt voor al het geduld om dat ook nog herhaaldelijk te bespreken en uit te leggen.

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Het laatste jaar op de kamer samen met Jeanne en Miriam. Dit bleek een zeer vruchtbare omgeving. Naast het feit dat de meeste stukken in dat jaar werden geschreven werden we ook alle 3 zwanger. Dat kan geen toeval zijn.

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Tenslotte, mijn oud CBG collega Henk Nab was degene die het idee opperde om het onderzoek bij Bruno Stricker te doen en dat was een goede keuze.

We must learn our limits. We are all something, but none of us are everything (Blaise Pascal).

De collega leden en adviseurs van de Gezondheidsraad commissies herziening RVP en antivirale middelen bij een grieppandemie wil ik bedanken voor alle discussies. Ik leer elke keer weer. De waarheid is een stuk breder dan het registratie dossier.

My colleagues at the EMEA vaccines working party, I want to thank you for all discussions, sharing of common interest and friendship. Especially, I want thank my dear friends and colleagues Ingrid Uhnoo and Stephania Salmaso for all the time you were willing to listen, discuss and comment on the various drafts of the revaccination studies. Roland Dobbelaer, thanks for all these years of trust both at EMEA and WHO and your willingness to act as opponent.

Dear Ivana Knezevic, I hope we will continue our WHO collaboration. It is always good to work with you and you had some great ideas.

Good friends, good books and a sleepy conscience: this is the ideal life (Mark Twain).

Antoinette, Mariët, Nicole en Josi bedankt voor al die vrijdagochtenden van ontspannen tennisballen slaan, frustraties wegmeppen en vooral veel kletsen en harten luchten.

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Sylvia, de vriendschap die 26 jaar geleden begon bij het hospiteren is altijd gebleven. Al die tijd bleef het als vanouds. Sommige dingen veranderen nooit.

Brigitte, 2 jaar waren kamergenoten. Samen schreven we het eerste projectvoorstel wat uiteindelijk tot dit proefschrift heeft geleid. Helaas ging je weg, maar gelukkig is de vriendschap gebleven. Bedankt dat je er was als het nodig was!

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The loneliest woman in the world is a woman without a close woman friend (George Santayana The Life of Reason, 1905-1906).

Verder heb ik de beste paranimfen die je kunt bedenken. Sabine en Inge, met jullie gaat deze dag gewoon lukken.

Sabine, samen den Haag, samen Rotterdam, samen promoveren, samen paranimfen en samen publiceren. Het was en is een belevenis samen met jou. Ik zit volgens jou altijd "on-line" maar jij antwoordt ook altijd direct. Al onze mailtjes met vragen, opmerkingen, frustraties, opbeurende verhalen en (soms smeuïge) commentaren zijn al een boek op zich. Bordeaux zal ik altijd blijven herkennen aan zijn winkelstraten.

Inge, al aan het begin van dit project heb ik je gevraagd paranimf te zijn om er zeker van te zijn dat je kwam. Deze dag is niet hetzelfde zonder jou erbij. Het blijkt dat voor echte vriendschap afstand niet relevant is. Bovendien, terwijl jij bestellingen aan het plaatsen was, liet ik SAS draaien en stuurde de eerste versie van het latere JAMA stuk uit. Niet iedere coauteur had door dat ook uit kaaswinkels manuscripten kunnen komen.

Om een groot verschil te willen maken, moeten we niet de kleine dagelijkse verschillen vergeten. Deze kunnen over de tijd optellen tot een groot verschil dat we niet op voorhand kunnen voorspellen (naar Marian Wright Edelman)

Ageeth, al jou zorg en inspanning voor de kinderen en ons, ik weet niet hoe ik je er in een paar woorden voor moet bedanken. Je bent uniek en we zijn je erg dankbaar. Ik heb nu meer tijd, beloof me, ga jij het nu ook rustiger aan doen?

> De ene generatie plant de boom, de volgende krijgt de schaduw (Chinees gezegde).

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> Like all the best families, we have our share of eccentricities, of impetuous and wayward youngsters and of family disagreements (Elisabeth II).

Gerrit, Cockie en Ans, jullie zien ook kleine zusjes worden groot. Gerrit, je bent m'n grote broer en grote voorbeeld. Cockie ik hoop dat je met Kees je geluk gevonden hebt. Ans ik bewonder je dynamiek en betrokkenheid.

> Ouders kunnen alleen goed advies geven of hun kinderen op het goede pad zetten, maar de vorming van hun karakter ligt in hun eigen handen (Anne Frank).

En dan nu de allerbelangrijkste en diegenen waar het uit eindelijk allemaal om gaat, Michaël, Laureanne en Kristien.

> Ik geloof in geluk en hoe harder ik werk hoe meer geluk ik heb (naar Thomas Jefferson).

Michaël wees trots op jezelf, in ieder geval ben ik heel trots op jou. Niemand doet je na.

> Je hebt chaos in je ziel nodig om een dansende ster geboren te laten worden (naar Nietzsche).

Laureanne jij kunt alles. Als jij je dromen volgt dan wordt het vast heel leuk.

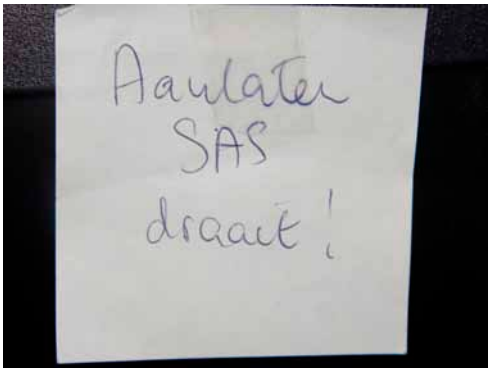
> Geluk zit in een klein hoekje (mama).

Kleine Kristien, als jij later net zo van het leven geniet als wij nu van jou, wordt het prachtig.

> Family life is full of major and minor crises -- the ups and downs of health, success and failure in career and marriage -- and all kinds of characters. It is tied to places and events and histories. With all of these felt details, life etches itself into memory and personality. It's difficult to imagine anything more nourishing to the soul (naar Thomas Moore).

Tenslotte Michiel. Zonder jou steun en je onvoorwaardelijke geloof in mij was dit nooit gelukt. Het waren bewogen jaren, maar het blijkt, samen kunnen we echt alles aan. Ik ben gelukkig met jou, trots op jou en trots op ons.

En nu is het de tijd voor leuke dingen. Michaël en Laureanne, in ieder geval hebben jullie de pc terug. Dit blaadje ligt in de prullenbak.



Curriculum vitae

Bettie Voordouw was born on February 28 1961 in Sittard, the Netherlands. She completed her Atheneum B in 1979 at the Serviam Lyceum in Sittard. Between 1979 and 1987 she studied Medicines at the State University Leiden. Following a one year working experience in clinical research at the department of Immunohaematology, Academic Hospital Leiden and five years at the department of Gynaecology and Obstetrics of the Erasmus Medical Center, Rotterdam, she joined the Medicines Evaluation Board in 1993, initially as clinical assessor for anti-infectives, with a main interest in vaccines. Since 2002 she is senior clinical assessor. Since its start in 2002, she is a member of the Vaccine Working Party of the EMEA. Furthermore she acts as advisor to the Health Council commission for the revision of the State Vaccination Program. In 1997 she obtained her Master of Public Health degree at the Netherlands School of Public Health, Utrecht.

In February 2001 she began the work described in this thesis at the department of Epidemiology and Biostatistics and the department of Medical Informatics at the Erasmus Medical Center in Rotterdam, in combination with her work at the Medicines Evaluation Board.

Bettie Voordouw is married to Michiel Lodewijks. Together they have 3 children, Michaël (1992), Laureanne (1995) and Kristien (2004).