Review

Do antibody responses to the influenza vaccine persist year-round in the elderly? A systematic review and meta-analysis

Barnaby Young a,⇑, Zhao Xiahong b, Alex R. Cook b,c, Christopher M. Parry d,e, Annelies Wilder-Smith a,f, Mark Chen I-Cheng a,b

a Institute of Infectious Diseases and Epidemiology, Tan Tock Seng Hospital, 11 Jalan Tan Tock Seng, 308433 Singapore, Singapore
b Saw Swee Hock School of Public Health, Tahir Foundation Building, National University of Singapore, 12 Science Drive 2, #09-01, 117549 Singapore, Singapore
c Yale-NUS College, National University of Singapore, 16 College Avenue West #01-220, 138527 Singapore, Singapore
d School of Tropical Medicine and Global Health, Nagasaki University Institute of Tropical Medicine, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan
e London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK
f Lee Kong Chian School of Medicine, 11 Mandalay Road, 308232 Singapore, Singapore

INTRODUCTION:
The influenza vaccine is less immunogenic in older than younger adults, and the duration of protection is unclear. Determining if protection persists beyond a typical seasonal epidemic is important for climates where influenza virus activity is year-round.

METHODS:
A systematic review protocol was developed and registered with PROSPERO [CRD42015023847]. Electronic databases were searched systematically for studies reporting haemagglutination-inhibition (HI) titres 180–360 days following vaccination with inactivated trivalent seasonal influenza vaccine, in adults aged ≥65 years. Geometric mean titre (GMT) and seroprotection (HI titre ≥1:40) at each time point was extracted. A Bayesian model was developed of titre trajectories from pre-vaccination to Day 360. In the meta-analysis, studies were aggregated using a random-effects model to compare pre-vaccination with post-vaccination HI titres at Day 21–42 (‘seroconversion’), Day 180 and Day 360. Potential sources of bias were systematically assessed, and heterogeneity explored.

RESULTS:
2864 articles were identified in the literature search, of which nineteen met study inclusion/exclusion criteria. Sixteen studies contained analysable data from 2565 subjects. In the Bayesian model, the proportion of subjects seroprotected increased from 41–51% pre-vaccination to 75–78% at seroconversion. Seroprotection subsequently fell below 60% for all serotypes by Day 360: A/H1 42% (95% CI 38–46), A/H3 59% (54–63), B 47% (42–52). The Bayesian model of GMT trajectories revealed a similar pattern. By Day 360, titres were similar to pre-vaccination levels. In the meta-analysis, no significant difference in proportion of subjects seroprotected from 41–51% pre-vaccination to 75–78% at seroconversion. Seroprotection subsequently fell below 60% for all serotypes by Day 360: A/H1 42% (95% CI 38–46), A/H3 59% (54–63), B 47% (42–52). The Bayesian model of GMT trajectories revealed a similar pattern. By Day 360, titres were similar to pre-vaccination levels. In the meta-analysis, no significant difference in proportion of subjects seroprotected, 0 (0.11, 0.11) or in log2 GMT 0.30 (0.02, 0.63) was identified by Day 360 compared with pre-vaccination. The quality of this evidence was limited to moderate on account of significant participant dropout.

CONCLUSIONS:
The review found consistent evidence that HI antibody responses following influenza vaccination do not reliably persist year-round in older adults. Alternative vaccination strategies could provide clinical benefits in regions where year-round protection is important.

© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
1. Introduction

Influenza is a common viral respiratory infection, which worldwide causes substantial morbidity and mortality, particularly at extremes of age [1,2]. Influenza vaccination is the primary tool available for disease control but immune responses to vaccination are reduced in the elderly compared with younger, healthy adults [3]. Persistence of vaccine-induced immunity over periods longer than a typical winter season have not been widely investigated, but similar to short term responses a reduced duration of persistence and hence protection against infection may be expected [4].

The primary immune response to the standard inactivated influenza vaccine is strain-specific antibody to surface haemagglutinin (HA) [5]. These antibodies mediate protection against infection by interfering with virus binding to host-cell receptors, and are measured with standardised Haemagglutination-Inhibition (HI) Assays [6]. Currently, age specific immunogenicity criteria based on the HI titre are used by the regulatory committees of the European Medicines Agency (EMA) and Federal Drug administration (FDA) [7]. For example, a HI titre of ≥1:40 is conventionally considered 'seroprotection', and more than 70% of younger adults, or 60% of older adults must reach this threshold for licensing.

A literature review published in 2008 studied antibody persistence in the elderly (>60 years) [8]. Up to 16 weeks post-vaccination the authors did not find evidence for substantial waning of seroprotection. This review was primarily qualitative and did not attempt to apply statistical methods to reported outcomes. Beyond this review, antibody persistence in the elderly has not been systematically assessed and a number of new studies providing data on antibody persistence have since been published.

Waning of vaccine effectiveness over the course of a winter season has been reported from a number of surveillance studies in Europe and Australia [9–11]. For example, in a study in Spain, vaccine effectiveness declined from 61% in the first 100 days after vaccination to 42% between days 100–119, and no protection after 120 days. This decline in effectiveness was most significant in the elderly aged over 65 years. These studies used the test-negative case-control design, and so do not include accompanying serological data. It is not clear to what extent this decline in effectiveness reflects loss of vaccine-induced immune responses, or reduced vaccine-strain matching from antigenic drift in circulating strains.

Limited data is available from studies of antibody persistence after influenza infection. An observational study monitored titre trajectories in subjects who were assessed to be infected with A/H1N1 during the 2009 pandemic (seroconversion without vaccination) [12]. In 71% haemagglutination-inhibition (HI) antibody titres were ≥1:40 immediately after the epidemic peak. This declined to 25% of subjects at 6 months, and only 14% at 1 year after the pandemic. In a sub-group analysis of the small number of elderly subjects in the cohort, the rate of antibody decline was significantly faster.

The duration of protection following vaccination is of particular public health importance in countries which report more than a single annual influenza season. Biannual epidemics, triannual epidemics and year round virus activity are described in tropical countries, from Indonesia and Malaysia to Peru and Mexico [13,14]. Despite the difference in seasonality, the burden of disease from influenza in countries with tropical, sub-tropical and temperate climates has been reported to be similar [15]. The implication of this differing epidemiology for vaccination schedules is yet to be understood. For example, recommendations for influenza vaccine timing from the World Health Organization (WHO) are based on the pattern of influenza virus activity rather than prospective studies of year-round vaccine effectiveness [16,17].

With year-round influenza virus activity in the tropics, year-round seroprotection is expected to be beneficial, but is least likely to be attained in populations such as the elderly with impaired immune responses. This study is a systematic review and meta-analysis of the available evidence for year-round persistence of vaccine-induced antibody following trivalent, inactivated, seasonal influenza vaccination in the elderly.

2. Materials and methods

An abbreviated study protocol is available from the National Institute for Health Research International Prospective Register of Systemic Reviews (PROSPERO), registration number CRD42015023847 [18]. The Preferred Reporting Items for Systematic Reviews and meta-Analyses (PRISMA) checklist for reporting of systematic review was also followed [19].

2.1. Search strategy and study selection

A search strategy was developed using the PICOST framework. Study inclusion and exclusion criteria are presented in Table 1.

| Population: Elderly ≥65 years |
|-------------------------------|
| Intervention: Trivalent inactivated seasonal influenza vaccination administered by intra-muscular injection |
| Comparison: No comparative group (e.g. healthy younger adults) will be included. HI antibody responses at selected time points will be compared with the pre-vaccination results. |
| Outcome: HI geometric mean titre (GMT) from 180 to 360 days after vaccination and proportion with GMTs ≥ 1:40 per Centre for Biologics Evaluation and Research (CBER) criteria |

Situation: For immunologic studies, the country in which the study is performed is not important.
Type of Study: Any study type - observational or randomised controlled trial (RCT).

A publication date limit of 1977 was chosen to reflect the year when a third co-circulating seasonal influenza strain (H1N1) was recognised and WHO recommendations switched from a bivalent to trivalent influenza vaccine [21]. The search construct developed for MEDLINE is available in the Supplementary Material. This was subsequently adapted for other information sources as required. Results were limited to human subjects and English language records.

Information sources searched to identify relevant literature included the MEDLINE, EMBASE and Global Health databases, the Cochrane Database of Systematic Reviews, Clinicaltrials.gov and reference lists of all included studies. After removal of duplicates, a three-stage screening process was used: reviewing title, then abstract and finally full text where possible.

2.2. Data collection

Data was extracted by a single reviewer using a template adapted from Cochrane.org. Data was collected as presented, with estimates of numerical values made where only graphical data was supplied. Verification of entered data was performed by an independent recollection of data from the source manuscript. Corresponding authors were contacted to request for further data or clarifications where necessary. Data that was not published in peer reviewed literature, but available in clinicaltrials.gov study results or study sponsor trial registries were included. Further systematic search for grey or unpublished literature was not performed. Seroprotection and GMT outcome data were collected for each influenza strain subjects were vaccinated with. Retention rates were calculated using the GMT data presented for A/H1.

2.3. Risk of bias and quality of evidence assessment

Risk of bias was assessed using the Cochrane Collaboration’s tools for assessing RCTs and non-randomised studies [22]. Each study was assessed as at either low, high or unclear risk for bias. The terminology for assessing risk of bias was harmonised for cohort and RCTs, however, bias assessments are not equivalent between scales. Studies were not excluded from the systematic review or meta-analysis as a result of this assessment.

The GRADE approach was used to evaluate the overall quality of evidence for each outcome [23]. Because the study compared pre- and post-vaccination results in the same subjects, both RCTs and observational studies started as ‘high quality’. They were downgraded by one level for serious (or by two for very serious) study limitations identified in the risk of bias assessment such as issues that may limit the generalisability of the reported results, inconsistency of results between studies, or potential publication bias.

2.4. Synthesis of results

Seroprotection and GMT were analysed by influenza subtype A/H1, A/H3 and type B at available time points. Number seroprotected (HI > 1:40) was estimated from provided data, rounded to integers. Where GMT confidence intervals (CI) were available, the sample standard deviation was calculated from the log2 transformed values and a Student’s t distribution assumed if not stated. Where only sub-group data was available from a study each subgroup was entered and analysed separately.

To account for both inter- and intra-study variability, Bayesian mixed-effect models were developed to determine an estimate of seroprotection and GMT for each subtype. We assumed a normal distribution for the GMT on a base-2 logarithmic scale and a binomial distribution for seroprotection for each subtype. The likelihood function of the GMT and seroprotection data could be then derived using the following models:

For GMT data, we assumed a log-Normal distribution

$$\log_{2}(GMT_{ij}) \sim N(\mu_{ij}, \sigma_{ij}^2)$$

$$\mu_{ij} = \alpha_i + \beta_j + \epsilon_i$$

where GMT_{ij} is the j th observed GMT in study i, \(\mu_{ij}\) controls the location of GMT\(_{ij}\) and \(\epsilon_i\) is the random effect term. Both \(\alpha_i\) and \(\beta_j\) are model parameters that need to be estimated where \(\alpha_i\) determines the study effect and \(\beta_j\) captures the variation in time.

For seroprotection data, we assumed a binomial distribution:

$$x_{ij} \sim Bin(p_{ij}, n_{ij})$$

$$\logit(p_{ij}) = \alpha_i + \beta_j + \epsilon_i$$

where \(x_{ij}\) is the j th observed number of subjects with GMTs > 1:40 in study i, \(n_{ij}\) is the j th observed sample size in study i, \(p_{ij}\) gives the estimate of seroprotection and \(\epsilon_i\) is the random effect term. Again, both \(\alpha_i\) and \(\beta_j\) are model parameters that need to be estimated where \(\alpha_i\) determines the study effect and \(\beta_j\) captures the variation in time.

Non-informative priors were adopted for model parameters. Parameters were estimated using Markov chain Monte Carlo
with 100,000 Metropolis-Hastings iterations with a thin of 10 iterations after a burn-in period of 50,000. Point estimates are posterior means and uncertainty intervals are 95% credible intervals (CrI).

The proportion of subjects seroprotected and log2 GMT were compared at baseline (pre-vaccination) with Day 21–42, Day 180 and Day 360 in the meta-analysis. The Mantel-Haenszel method was used to calculate the risk difference in seroprotection. Mean differences in log2 GMT were aggregated by inverse-variance weighting. Random-effects models were used for all meta-analyses to reflect the assumption that the effects being estimated in the different studies are not identical. Sub-group analyses by influenza subtype were also performed (A/H1, A/H3, B).

Variability of outcomes between studies was assessed using the heterogeneity statistic $I^2$. Possible publication bias was assessed by visual interpretation of funnel plots.

Descriptive statistics were calculated using Microsoft Excel 2016 (Microsoft Corporation, Richmond, USA). The Bayesian binomial/normal model was implemented in R version 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria). Meta-analyses were calculated using RevMan v5.3 (The Cochrane Collaboration, Copenhagen, Denmark). Statistical significance was assumed with a 2-sided alpha of 0.05.

3. Results

3.1. Study selection and characteristics

Database searches were performed in September 2015. The PRISMA flow diagram for the study selection process and reasons for exclusion of studies at full-text screening are shown in Fig. 1. Summary characteristics of the included studies are shown in Table 2. There were fifteen randomised control trials (RCTs) and 4 cohort studies.

Fourteen of the RCTs compared standard dose inactivated trivalent influenza vaccine with alternative formulations (adjuvanted, high-dose vaccine, intra-dermal vaccine or quadrivalent vaccine). One RCT compared the effect of an oral adjuvant with placebo. Three RCTs included an immunogenicity analysis as a subset of a larger analysis with clinical endpoints, while the others were designed with immunological primary endpoints. One of the cohort studies was designed to investigate antibody persistence in the elderly and other age groups as the primary objective. Two cohort studies investigated serological responses to influenza vaccination after first and subsequent annual vaccination, while one was an observational study of booster influenza vaccine at 12 weeks in elderly travellers.
Retention fell to 68.2% from Day 1 to 360 for two of the five studies reporting this data.

In sixteen studies the number of subjects enrolled was available. 20.7% (677/3269) of subjects were excluded with no serological data presented. It could not be evaluated if exclusion of these subjects introduced a systematic bias in results.

### 3.2. Risk of bias within studies

Overall nine RCTs were considered at low risk for bias, three at high risk and for three the risk was unclear (see Supplementary Material for full assessment). One cohort study was considered at low risk of bias, two at high risk and for one the risk was unclear.

Six RCTs were conducted as open-label, eight observer-blinded, and one double-blind. Although open-label studies have a greater potential for bias when compared to blinded studies, open-label studies were not assessed as high-risk on the basis of this because of the immunological nature of outcomes in this review. Most of the RCTs failed to report their allocation concealment methodology, but as this review is not comparing outcomes between groups, this limitation was not considered likely to cause significant bias.

For both RCT and cohort studies the major potential source of bias was subject dropout. For three RCTs and two cohort studies, results were selectively reported, excluding subjects who did not have HI data for Day 180–360, or the enrolled sample size was not clear. Two of the fourteen evaluable studies reported loss of >10% of subjects from Day 1 vaccination to Day 180. The overall retention rate from these thirteen studies at Day 180 was 95.1%.

### 3.3. Systematic review and Bayesian models of antibody persistence

The pattern of GMT and seroprotection rise and decline after vaccination was similar across all studies. Raw data extracted is available in the Supplementary Material. The majority of individual studies demonstrated a good initial serological response to vaccination with waning from seroconversion to Day 360. The degree of pre-vaccination immunity and magnitude of post-vaccination responses varied considerably. From individual studies, only one of 11 seroprotection and three of 14 GMT comparisons demonstrated statistically significant higher levels at Day 360 compared with pre-vaccination.

Summary data from the Bayesian model of antibody persistence is presented in Table 3. The estimate of population seroprotection was just above the CBER licensure threshold of 60% at Day 180, and by Day 360 fell below this threshold for all three serotypes.
3.4. GMT meta-analysis

Fifteen studies reported the pre-vaccination GMT and were included in the meta-analysis. All fifteen provided data for comparison at Day 21–42, fourteen for comparison at Day 180, and four for Day 360.

In the overall analysis, combining estimates for influenza A/H1, A/H3 and B, log2 GMT difference declined linearly from Day 21–42 to Day 360, implying an exponential decay in GMT titres (Fig. 2). GMTs at Day 360 were not significantly different from pre-vaccination (0.30, 95% CI 0.02, 0.63). This result was consistent when analysed by subtype A/H3 (0.47, 95% CI 0.17, 1.15) and type B (0.27, 95% CI 0.04, 0.51). While a significant difference at Day 360 was detected for A/H1 (0.57, 95% CI 0.23, 0.91), the overall trend was similar.

3.5. Seroprotection meta-analysis

Thirteen studies which reported the proportion of subjects who were seroprotected pre-vaccination were included in the meta-analysis. All provided data for comparison at Day 21–42, twelve for comparison at Day 180, and three for Day 360.

Similar to the analysis of GMTs, when combining influenza A/H1, A/H3 and B, seroprotection risk difference compared with pre-vaccination declined linearly from Day 21–42 to Day 360 (Fig. 2), and no significant difference in seroprotection compared with pre-vaccination was detected by Day 360 (0, 95% CI –0.11, 0.11). Analysis by individual influenza subtypes A/H1, A/H3, and type B were consistent, with no significant difference from the pre-vaccination level for each at Day 360: A/H1 (0.09, 95% CI –0.15, 0.33), A/H3 (–0.08, 95% CI –0.11, 0.12), B (0, 95% CI –0.13, 0.13).

3.6. Risk of bias between studies

Substantial heterogeneity between studies was evident in the main pooled analysis from the heterogeneity statistic I² (89–98%). This estimate of heterogeneity was generally lower for the Day 360 and individual subtype analyses.

No asymmetry was evident from examination of the funnel plots for seroprotection risk difference at all time points. For GMT mean difference, asymmetry was evident in the Day 21–42 funnel plots (Fig. 3). Asymmetry was primarily a result of a larger than expected mean difference (MD) from four data points, all provided by A/H1 and A/H3 from study 13 (Tinoco et al.). Funnel plots were similar for Day 180, while no asymmetry was apparent at Day 360 (see Supplementary Material).

3.7. Quality of evidence

Estimates of differences between GMT and seroprotection pre-vaccination and Day 360 were assessed to be of moderate-

### Table 3

| Subtype | Pre-vaccination | Day 21–42 | Day 180 | Day 360 |
|---------|-----------------|-----------|---------|---------|
|         | GMT (95% Credible Intervals) |           |         |         |
| A/H1    | 20.3 (18.8–22.0) n = 2537 | 65.8 (60.5–71.0) n = 2536 | 33.6 (31.1–36.5) n = 2327 | 22.6 (19.8–26.4) n = 734 |
| A/H3    | 23.6 (21.7–25.6) n = 2538 | 83.3 (76.1–90.5) n = 2537 | 45.3 (41.6–49.5) n = 2327 | 36.0 (30.9–41.4) n = 734 |
| B       | 32.0 (29.2–35.3) n = 2470 | 78.2 (71.5–86.2) n = 2469 | 54.9 (49.9–60.1) n = 2327 | 28.2 (23.1–34.1) n = 666 |

### Notes

Pre-vaccination was detected by Day 360 (0, 95% CI –0.11, 0.11). Analysis by individual influenza subtypes A/H1, A/H3, and type B were consistent, with no significant difference from the pre-vaccination level for each at Day 360: A/H1 (0.09, 95% CI –0.15, 0.33), A/H3 (–0.08, 95% CI –0.11, 0.12), B (0, 95% CI –0.13, 0.13).

### Figures

**Fig. 2.** Overall and sub-group analysis by influenza subtype of mean difference in log2 GMT and seroprotection pre-and post-vaccination at each time point (A + C), and the forest plots for Day 360 (B + D).
quality. Despite heterogeneity in vaccine responses initially, study results were consistent by Day 360. This assessment was considered as only moderate-quality evidence because of problems with incomplete reporting of outcome data following subject dropout. The quality of evidence for the effect size estimates of the change in GMT and seroprotection compared between pre-vaccination at The quality of evidence for the effect size estimates of the change in GMT and seroprotection compared between pre-vaccination at Day 21–42 and Day 180 were assessed as low due to heterogeneity.

4. Discussion

This meta-analysis finds robust evidence to support the conclusion that in the elderly over than 65 years, haemagglutination-inhibition (HI) antibody responses following influenza vaccination does not reliably persist year-round. The findings were consistent between the Bayesian model and the meta-analysis, and consistent when using seroprotection (HI ≥ 1:40) or GMT as the endpoint. Overall, an exponential decay in antibody titres was evident from Day 21–42 to Day 360 after vaccination, as reflected in a linear decline in the estimates of mean difference in log$_2$ GMT. By Day 360, GMTs and the proportion of subjects seroprotected were not significantly different from pre-vaccination levels, and for seroprotection, below the CBER licensing threshold.

There is a lack of clinical studies on vaccine efficacy or effectiveness from elderly populations in tropical countries to confirm the clinical significance of this finding. However, the immunological evidence presented here suggests that the elderly in countries with biannual and year-round influenza epidemics would benefit from an alternative to the currently recommended annual standard dose trivalent inactivated influenza virus (IIV3). Newer vaccine formulations which include an immunological adjuvant - MF59 or AS03 - or a higher antigen dose are more immunogenic than standard IIV3 [49,50]. Over a winter season these have been reported to reduce infection rates compared with IIV3 in double-blind RCTs, but it is not clear if this would translate into longer term benefits [37,51]. For IIV3 with adjuvant, serological outcome data is mixed at Day 180, with reports of significantly higher HI titres compared to standard IIV3 in some studies [40,44,46]. Any serological benefit, however, does not appear to persist to Day 360 [24,37,43]. Antibody persistence data beyond Day 180 is not currently available for the high dose vaccine. An alternative approach to changing the formulation may be to vaccinate more regularly – for example before each season in countries with biannual epidemics. The outcomes of such a strategy has not been investigated.

Despite the established use of a HI titre of ≥ 1:40 to define seroprotection, this threshold is associated with only 40–70% protection from infection in the elderly and younger adults [52,53]. Using this threshold will underestimate the changes in proportion of subjects who become susceptible to infection when GMTs decline. Coudeville et al. developed a protection curve to model the probability of protection at different HI titres [54]. As the HI titre increases from 1:40 to 1:100, the probability of protection increases to approximately 90%, with marginal benefits beyond this. Thus, even if vaccine responses meet the ‘seroprotection’ cut-off of 1:40 at Day 180, decay in titres in the elderly may result in a substantial proportion of the population becoming susceptible to infection, including before the end of a seasonal epidemic in temperate countries. Clearly, if following seroconversion significant susceptibility to infection develops by Day 180, this is only likely to increase further by Day 360 and offer inadequate efficacy with an annual vaccination strategy for elderly individuals in the tropics.

The persistence of other immune responses to vaccination also needs to be considered. Microneutralization (MN) assays may be a more accurate measure of humoral immunity than the HI assay, and MN titres also correlate with protection against influenza infection [55,56]. Cell-mediated immunity reduces the severity of disease after infection, and while this is only weakly induced by IIV3 it may still have a clinically significant role [57]. Vaccine-induced T-cell responses have been reported to be better correlates of protection than HI titres in the elderly [58]. This review did not seek to identify studies which explored persistence of these immune responses in the elderly after influenza vaccination, but this would be useful to investigate.

There was substantial variability in baseline GMTs, seroprotection levels, and the magnitude of vaccination responses between studies. Some of this variability is influenza strain dependent, and reflects the immunogenicity of individual strains, and host immunological memory from previous infection or vaccination exposure. The influence of pre-existing immunity on vaccine responses is complex. An observational study of mainly younger adults and children suggested interference in vaccine responses...
from previous vaccination [59]. However, a meta-analysis identi-
ified high pre-vaccination titres and previous vaccination as predic-
tors of higher vaccine responses [60]. Influenza vaccination rates in
the year prior to study enrolment were high in those studies that
reported this (70–100%), while information on recent influenza
infections were not available.

Influenza independent mechanisms which will affect vaccine
response in the elderly include participant health, concomitant
medications, and in particular the impact of immunosenescence.
RCTs generally excluded participants with unstable health condi-
tions or immunosuppression due to the presence of specific co-
morbidities or treatment with cytotoxic chemotherapy or high-
dose corticosteroids. Most studies also recruited volunteers from
the community rather than long-term care facilities. It is not clear
if the prevalence and severity of common chronic diseases such as
diabetes mellitus and chronic obstructive pulmonary disease in
recruited subjects is similar to the general population. While these
conditions are associated with increased morbidity and mortality
after influenza infection, their impact on vaccine responses is less
certain [61–63]. In a study of antibody persistence after vaccina-
tion among subjects >50 years neither age nor presence of chronic
disease were significantly associated with maintenance of sero-
protection at 8 months [64]. Song et al. also did not find the presence
of chronic diseases as a significant risk factor for reduced antibody
 persistence at 12 months [4].

Immunosenescence describes the complex process of immune
dysregulation that develops with aging, but defining immunose-
nescence by a single variable – age – as is traditional in vaccina-
tion guidelines, is an over-simplification [65]. The pace of
immunosenescence development is the result of numerous inter-
actions between host genetic and environmental processes which
also includes biological sex, nutritional status, chronic stress,
chronic infection, and other co-morbidities. An important sys-

temic cause and consequence of immunosenescence may be the
chronic release of inflammatory cytokines such as TNF, IL-1 and
IL-6 [66]. This syndrome of ‘inflammaging’ correlates with pro-
gression of a number of chronic diseases and contributes to func-
tional decline and frailty in the elderly. In vitro studies have also
implicated inflammmaging as associated with reduced influenza
vaccine response [67].

It is debatable what age cut-off is most appropriate for this
review – the older adult immunogenicity criteria used by the
FDA apply when age is ≥65 years, while for the EMA the cut-off
is >60 years. A number of studies were excluded from this review
because they enrolled younger subjects. Lowering the inclusion
criteria to >60 years would probably not have significantly altered
its findings, although we can expect that vaccine responses would
have been better and more durable with the addition of younger
individuals. Deeper understanding of the impact of age and other
risk factors for immunosenescence was beyond the scope of this
review, but could be further explored if patient level data were
available.

The findings of this meta-analysis were consistent when anal-
ysed by GMT or seroprotection, and in both the overall analysis
or by influenza subtypes. The variability between subjects and
studies places limits on applying the estimates of the magnitude
of changes in GMT and seroprotection to individual patients or
influenza strains. Some of this variability is mitigated by the
matched design of the meta-analysis, with control and experimen-
tal outcomes measured in the same population. There was a signif-

icant loss of participants by Day 360 in all the studies included in
the meta-analysis. Reasons for dropout were generally not avail-
able, but participant morbidity and mortality is likely to be an
important contributor. This is likely to bias study results towards
more sustained immune responses than the general elderly
population.

Identification of studies in this review may have missed rele-
vant studies, through human error, imperfections in the literature
databases or search methodology, or a failure to identify unpub-
lished studies. As relatively few studies included evaluable data
at Day 360, this could impact findings of the statistical analysis.
However, more than 2000 individual comparisons were available
for analysis at Day 360, and so new data is less likely to impact
the overall trend of antibody decline after seroconversion.

In conclusion, the decline from Day 21–42 to 360, in GMTs and
the proportion of subjects seroprotected suggests that clinical pro-
tection is not likely to persist year-round in the elderly. Confirma-
tion of this finding with studies of vaccine effectiveness is
necessary, but identifying alternative vaccination schedules which
provide year-round protection against influenza infection is likely
to offer substantial public health benefit, particularly in tropical
countries with year-round virus activity.

Conflicts of interest

BY has received research funding from Sanofi Pasteur. All other
authors have no potential conflicts of interest to report.

Funding

BY was supported by the National Healthcare Group Thematic
Grant (NTG/13007); XZ and ARC were supported by the Centre
for Infectious Diseases Epidemiology and Research (CIDER); and
ARC was also supported by the Ministry of Health -Singapore
(Communicable Disease Public Health Research Grant
CDPHRG12NOV021).

The funding agencies had no involvement in the conduct of the
research or preparation of this article.

Appendix A. Supplementary material

Supplementary data associated with this article can be found,
in the online version, at http://dx.doi.org/10.1016/j.vaccine.2016.11.
013.

References

[1] Nair H, Brooks WA, Katz M, Roca A, Berkley JA, Madhi SA, et al. Global burden
of respiratory infections due to seasonal influenza in young children: a
systematic review and meta-analysis. Lancet Lond Engl 2011;378:1917–30.
http://dx.doi.org/10.1016/S0140-6736(11)6051-9.

[2] Thompson WW, Comanor L, Shay DK. Epidemiology of seasonal influenza: use
of surveillance data and statistical models to estimate the burden of disease. J
Infect Dis 2006;194(Suppl. 2):S82–91. http://dx.doi.org/10.1086/509758.

[3] Jefferson T, Di Pietrantonj C, Al-Ansary LA, Feronni E, Thorning S, Thomas RE.
Vaccines for preventing influenza in the elderly. Cochrane Database Syst Rev
2010:CD004876. http://dx.doi.org/10.1002/14651858.CD004876.pub.

[4] Song JF, Cheong HJ, Hwang B, Choi WS, Jo YM, Park DW, et al. Long-term
immunogenicity of influenza vaccine among the elderly: risk factors for poor
immune response and persistence. Vaccine 2010;28:3929–35. http://dx.doi.
org/10.1016/j.vaccine.2010.03.067.

[5] Ohmit SE, Petrie JS, Cross RTS, Johnson E, Monto AS. Influenza
hemagglutination-inhibition antibody titer as a correlate of vaccine-induced
protection. J Infect Dis 2011;204:1879–85. http://dx.doi.org/10.1093/infdis.
jii663.

[6] Brandenburg B, Koudstaal W, Goudsmit J, Klaren V, Tang C, Buijn MV, et al.
Mechanisms of hemagglutinin targeted influenza virus neutralization. PLoS
One 2013;8:e80034. http://dx.doi.org/10.1371/journal.pone.0080034.

[7] Wijnans L, Voordouw B. A review of the changes to the licensing of influenza
vaccines in Europe. Influenza Other Respir Viruses 2016;10:2–8. http://dx.doi.
org/10.1011/jnr.1255.

[8] Skowronska DM, Tweed SA, De Serres G. Rapid decline of influenza vaccine-
induced antibody in the elderly: is it real, or is it relevant? J Infect Dis 2008;
197:490–502. http://dx.doi.org/10.1086/524146.

[9] Sullivan SM, Konadina N, Grant K, Jelley L, Papadakis G, Kelly H. Influenza
vaccine effectiveness during the 2012 influenza season in Victoria, Australia:
influences of waning immunity and vaccine match. J Med Virol 2014;86:1017–25.
http://dx.doi.org/10.1002/jmv.23847.
responses for evaluating the seasonal influenza vaccine. J Virol Methods 2012;182:43–9. http://dx.doi.org/10.1016/j.jviromet.2012.03.004.

[56] Verschoor CP, Singh P, Russell ML, Bowdish DME, Brewer A, Cyr L, et al. Microneutralization assay titres correlate with protection against seasonal influenza H1N1 and H3N2 in children. PLoS One 2015;10:e0131531. http://dx.doi.org/10.1371/journal.pone.0131531.

[57] Li CK, Rappuoli R, Xu X-N. Correlates of protection against influenza infection in humans—on the path to a universal vaccine? Curr Opin Immunol 2013;25:470–6. http://dx.doi.org/10.1016/j.coi.2013.07.005.

[58] McElhaney JE, Xie D, Hager WD, Barry MB, Wang Y, Kiepeinger A, et al. T cell responses are better correlates of vaccine protection in the elderly. J Immunol Baltim Md 1950;2006(176):6333–9.

[59] Ohmit SE, Petrie JG, Malosh RE, Fry AM, Thompson MG, Monto AS. Influenza vaccine effectiveness in households with children during the 2012–2013 season: assessments of prior vaccination and serologic susceptibility. J Infect Dis 2015;211:1519–28. http://dx.doi.org/10.1093/infdis/jiu650.

[60] Beyer WE, Palache AM, Sprenger MJ, Hendriksen E, Tukker JJ, Darioli R, et al. Effects of repeated annual influenza vaccination on vaccine sero-response in young and elderly adults. Vaccine 1996;14:1331–9.

[61] Nath KD, Burel JC, Shankar V, Pritchard AH, Towers M, Looke D, et al. Clinical factors associated with the humoral immune response to influenza vaccination in chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis 2014;9:51–6. http://dx.doi.org/10.2147/COPD.S53590.

[62] Sheridan PA, Paich HA, Handy J, Karlsson EA, Schultz-Cherry S, Hudgens M, et al. The antibody response to influenza vaccination is not impaired in type 2 diabetics. Vaccine 2015;33:3306–13. http://dx.doi.org/10.1016/j.vaccine.2015.05.041.

[63] Gross PA, Quinnan GV, Weksler MS, Setia U, Douglas RG. Relation of chronic disease and immune response to influenza vaccine in the elderly. Vaccine 1989;7:303–8.

[64] Talbot HK, Coleman LA, Zhu Y, Spencer S, Thompson M, Cheng P-Y, et al. Factors associated with maintenance of antibody responses to influenza vaccine in older, community-dwelling adults. BMC Infect Dis 2015;15:195. http://dx.doi.org/10.1186/s12879-015-0926-8.

[65] Goronzy JJ, Weyand CM. Understanding immunosenescence to improve responses to vaccines. Nat Immunol 2013;14:428–36. http://dx.doi.org/10.1038/ni.2588.

[66] Baylis D, Bartlett DB, Patel HP, Roberts HC. Understanding how we age: insights into inflammaging. Longev Heal 2013;2:8. http://dx.doi.org/10.1186/2046-2395-2-8.

[67] Frasca D, Diaz A, Romero M, Landin AM, Blomberg BB. High TNF-α levels in resting B cells negatively correlate with their response. Exp Gerontol 2014;54:116–22. http://dx.doi.org/10.1016/j.exger.2014.01.004.