Influenza vaccination as a novel means of preventing coronary heart disease: Effectiveness in older adults

Amal Aidoud, Julien Marlet, Denis Angoulvant, Camille Debacq, Gaetan Gavazzi, Bertrand Fougère

1 # Influenza vaccination as a novel means of preventing

2 # coronary heart disease: effectiveness in older adults

3

4 **Amal Aidoud[a*], Julien Marlet[b], Denis Angoulvant[c], Debacq Camille[a], Gaëtan Gavazzi[d],**

5 **Bertrand Fougère[a,e]**

6

7 *[a]Division of Geriatric Medicine, Tours University Hospital, Tours, France*

8 *[b]French National Institute of Health and Medical Research INSERM U1259, Tours, France, University of Tours,*

9 *Tours, France*

10 *[c] Cardiology Unit, Trousseau Hospital, CHRU de Tours & EA4245, Loire Valley Cardiovascular collaboration,*

11 *Tours University, Tours, France*

12 *[d]University Clinics of Geriatrics, University Hospital of Grenoble-Alpes, GREPI EA7408 University of Grenoble*

13 *Alpes, Grenoble, France*

14 *[e]Éducation, éthique, santé (EA 7505), Tours University, Tours, France*

15

16 [*] Corresponding author at: Médecine Gériatrique, Centre Hospitalier Régional Universitaire

17 de Tours, Hôpital Bretonneau, 2 Boulevard Tonnellé, F-37000 Tours, France.

18 Tel.: +33-247-473-713; Fax: +33-234-389-536

19 E-mail: amal.aidoud@univ-tours.fr (A. Aïdoud).

20

1

Abstract

Atherosclerosis can have various etiologies, including several newly recognized immunoinflammatory mechanisms. A growing body of evidence suggests that influenza infection is chronologically linked to acute myocardial infarction (AMI), and thus that the virus is a novel cardiovascular disease (CVD) risk factor. Morbidity and mortality rates for both influenza infection and AMI rise markedly with age. Epidemiological studies have demonstrated that influenza vaccination (IV) has a cardioprotective effect, especially in people aged 65 and over; hence, IV may be of value in the management of CVD. These observations justify efforts to better understand the underlying mechanisms and to identify therapeutic targets in older adults.

In view of the above, the objective of the present study was to review the literature data on the cellular mechanisms that link IV to the prevention of atherosclerotic complications. Given the greater burden of CVD in older subjects, we also questioned the impact of aging on this association.

The most widely recognized benefit of IV is the prevention of influenza infection and the latter's cardiovascular complications. In a new hypothesis, however, an influenza-independent effect is driven by vaccine immunity and modulation of the ongoing immunoinflammatory response in individuals with CVD. Although influenza infection and IV both induce a proinflammatory response, they have opposite effects on the progression of atherosclerosis – suggesting a hormetic phenomenon.

Aging is characterized by chronic inflammation (sometimes referred to as "inflammaging") that progresses insidiously during the course of aging-related diseases, including CVD. It remains to be determined whether vaccination has an effect on aging-related diseases other than CVD. Although the studies of this topic had various limitations, the results highlight the potential benefits of vaccination in protecting the health of older adults, and should drive research on the

molecular immunology of the response to IV and its correlation with atheroprotective processes.

Key words: atherosclerosis; immunity; vaccination; influenza; older adults

Abbreviation: ACS: acute coronary syndrome; AMI: acute myocardial infarction; ApoB-100: apolipoprotein B-100; BKB2R: bradykinin B2 receptor; CHD: coronary heart disease; CRP: C-reactive protein; CVD: cardiovascular disease; HA: hemagglutinin; HF: heart failure; 25-hydroxyvitamin D: 25(OH) D, IL: interleukin; IV: influenza vaccination; ox-LDL: oxidized low-density lipoprotein;; NO: nitric oxide; TNF-α: tumor necrosis factor-alpha,

Introduction

Atherosclerosis is an immunoinflammatory disease that is responsible for cardiovascular disease (CVD). Epidemiological data show that CVD is a major burden in older adults with regard to mortality, functional decline, and economic impact. In 2016, CVD was the leading cause of death in older adults – accounting for 42% of all deaths in that age group [1]. Most CVD-related mortality is due to ischemic heart disease and stroke. According to epidemiological projections, population aging will increase the CVD burden – attesting to a true public health challenge.

After CVD, respiratory tract infection (including influenza) is the third-ranked cause of death worldwide. It has been estimated that each year, 290,000 to 650,000 deaths worldwide are influenza-related [2]. A growing body of epidemiological and biological data suggests that influenza infection is a risk factor for coronary heart disease (CHD). The elevated cardiovascular mortality in winter and its association with the influenza disease peak [3], the local and systemic inflammatory mechanisms induced by the virus [4–7], and the virus's selective presence in atherosclerotic lesions in animal and human vessels [4,5] have prompted researchers to consider the influenza virus as a risk factor for atherosclerosis progression. The possibility that the influenza virus triggers CHD complications has prompted a resurgence of interest in influenza vaccination (IV). Secondary prevention studies of older adults have shown that IV is associated with a reduction in the incidence of major adverse cardiovascular events [8–13]. Thus, IV might be of particular value in older adults at a high risk of influenza infection and CHD complications. Understanding the underlying cellular processes is likely to be crucial in the development of new strategies for preventing and/or treating cardiovascular complications.

In the present study, we reviewed the literature on cellular mechanisms that might explain the IV's atheroprotective effect on CVD. Hence, our goals were to provide an overview of the putative atheroprotective effect of IV in older adults and to suggest potentially appropriate treatment approaches and research perspectives.

1   Cardiovascular disease and aging

1.1   *Atherosclerosis*

Atherosclerotic processes result in profound, progressive changes in the arterial wall. These changes are inflammatory in nature, and so atherosclerosis can be considered as a chronic inflammatory disease driven by both the innate immune system (via macrophages) and the adaptive immune system (via T lymphocytes) (for a review, see [14–16]).

Endothelial dysfunction is an important, early event in atherogenesis. Alterations in the endothelium enabled low-density lipoprotein (LDL) to permeate more readily into the intima. Trapped LDLs undergo oxidation *in situ*. The oxidized LDL (ox-LDL) chemo-attracts monocytes and T cells. The monocytes differentiate into macrophages, internalize ox-LDL via unregulated receptors and, lastly, become foam cells. The accumulation and proliferation of immune cells in the vessel wall is associated with the release of cytokines, chemokines, and other vasoactive molecules. This chronic inflammatory response subsequently stimulates the migration and proliferation of smooth muscle cells from the media to the intima, resulting in the formation of an intermediate lesion. If this inflammatory process continues, the intermediate lesion becomes a mature plaque with a core rich in lipids and necrotic cells and surrounded by a collagen-rich, fibrous cap. Proteases secreted by the macrophages and T cells degrade the

5

collagen, which destabilizes the plaque and, ultimately, leads to its rupture. Lastly, exposure of the blood to procoagulant factors released by the ruptured plaque triggers thrombosis and ischemia.

1.2 Arterial aging

Arterial aging is a key factor in the incidence and prevalence of CVD. It leads to structural, functional, and mechanical arterial remodeling, even when other CVD risk factors are absent. Although arterial aging is a normal physiological process, aged arteries are characterized by endothelial dysfunction, vascular remodeling, inflammation, calcification, and increased stiffness – all of which are also characteristics of atherosclerosis. Aged vessels express elevated levels of pro-inflammatory mediators and take up plasma LDL more readily. These effects may result in greater expression of leukocyte adhesion molecules on endothelial cells in aged vessels, which would induce monocyte migration, greater uptake of atherogenic lipoproteins, and subsequent inflammation – all key events that ultimately promote atherosclerosis **[17,18]**. The direct relationship between aging and vascular health is also evident in progeria, a syndrome in which patients present accelerated aging – including accelerated atherosclerosis and premature cardiovascular mortality **[19]**. The boundaries between arterial aging and atherosclerosis are difficult to establish; hence, these two entities should be considered on a continuum, with synergistic actions on the cardiovascular risk.

2   Cardiovascular disease, inflammation, and the influenza virus.

Scientific research has shown that atherosclerosis is a multifactorial disease. Epidemiological investigations have evidenced associations with various conventional risk factors, on which

today's treatments for atherosclerosis are focused. However, the body of data on the involvement of infections (including the influenza virus) in the atherosclerosis process is growing.

A link with the infectious burden was first evoked in 1978 by Fabricant, who observed "*athero-arteriosclerosis [damage] closely resembling that in humans*" in chickens infected by gallid herpes virus 2 (Marek's disease virus) [20]. Subsequent clinical and animal studies revealed an association between CVD and various pathogens – initially *Chlamydia pneumoniae* and then *Helicobacter pylori*, *Porphyromonas gingivalis*, and others [21]. These data have stimulated great interest in the infectious hypothesis because of the prospect of prevention.

2.1 *The influenza virus and atherosclerosis*

An association between influenza infection and acute coronary syndrome (ACS) was first suggested during the influenza epidemic that struck Europe and the United States in the early 1900s. During these epidemics, more than half of all deaths were attributed to a cause other than influenza, and CVD was predominant [22]. The excess cardiovascular mortality in winter and its association with the influenza peak strongly suggested a relationship between influenza infection and ACS [3]. In a large cohort of older adults, the risk of myocardial infarction and stroke were found to be significantly higher during the first three days following the diagnosis of a respiratory infections (incidence ratio for ACS, 4.95; 95%CI [4.43-5.53] and for stroke, 3.19; 95%CI [2.81 to 3.62]) [23]. More recently, influenza infection has been linked to a higher risk of cardiovascular mortality within 14 days, and was strongly correlated with fatal ACS (with an increase in incidence ranging from 5.8% (95%CI [2.5-9.1%]) to 13.1% (95%CI [5.3-20.9%]) [24].

163 Studies of patients with laboratory-confirmed influenza infections have underlined the virus's

164 involvement in the atherosclerotic process. In one study, serologically confirmed influenza

165 infection was significantly more frequent in patients with recent ACS [25]; however, this was

166 not a predictive factor in MacIntyre et al.'s case control study [26]. A recent self-controlled

167 case-series (median patient age: 77 years) revealed that laboratory-confirmed infection was

168 associated with a 6-fold higher risk of hospital admission for ACS [27]. Although many

169 pathogens have been studied as putative risk factors in CHD, the epidemiological data suggest

170 that the influenza virus is the most serious candidate for involvement in the atherosclerotic

171 process.

172

173 *2.2 Acute coronary syndrome triggered by the influenza virus*

174 The results of animal studies have provided a pathophysiological explanation for the influenza

175 virus's putative involvement in the atherosclerotic process. Influenza infection might trigger

176 ACS in one or more of three ways: (i) *a direct viral effect* of the virus present in the

177 atherosclerotic plaque, (ii) *an indirect inflammatory effect* due to a systemic inflammatory

178 response to infection, and (iii) *a direct cross-reactive effect,* related to cross-reactions between

179 viral antigens and proatherogenic plaque components (**Figure 1**).

180 The presence of a **direct effect** is suggested by the virus's vascular tropism and its association

181 with lesion progression. In the ApoE$^{-/-}$ mouse model of atherosclerotic disease, influenza A

182 virus was found to infect and specifically reside in fibro-lipid plaques – even in the absence of

183 apparent viremia [4]. Furthermore, the presence of the virus was associated with an exacerbated

184 local inflammatory response involving macrophage infiltration and a systemic pro-

185 inflammatory cytokine response. Furthermore, ApoE$^{-/-}$ mice inoculated with a lethal dose of

186 influenza A virus showed a significant increase in the numbers of smooth muscle cells and

187 inflammatory cells, the presence of platelet aggregates, and the occasional formation of

188 occlusive thrombi in atherosclerotic plaques - a set of changes also found after fatal ACS [5].

189 Similarly, influenza virus can infect and modulate human endothelial cells *in vitro*; the infected

190 cells took on a procoagulant profile [7] and became more susceptible to apoptosis [6]. Lastly,

191 virus-induced secretion of pro-inflammatory cytokines might also stimulate degradation of the

192 extracellular matrix [28]. These virus-related actions might make atherosclerotic lesions more

193 likely to rupture. Exposure of destabilized, procoagulant plaques to the blood would then result

194 in the formation of intravascular thrombi.

195 The **indirect effect** is linked to a systemic inflammatory response to infection. Regardless of

196 effects found *in situ*, the response to infection induces acute inflammation. This state is

197 characterized by the massive production of pro-inflammatory cytokines, oxidative stress

198 mediators and vasoactive molecules by the infected pulmonary cells and then the release of

199 these molecules into the circulation [29]. This immune response targets the infection site and

200 then eliminates the virus. Atherosclerosis progression is characterized by a chronic

201 immunoinflammatory process, involving the same mediators as in an influenza infection. Thus,

202 this systemic inflammatory secretion in response to infection will have a remote effect on the

203 atheroma plaque. In the ApoE$^{-/-}$ mouse, significant inflammatory and thrombotic effects are

204 observed between 7 and 10 days after infection by the influenza A virus. These effects include

205 the massive attraction of immune cells (including monocytes and T cells), their adhesion to and

206 infiltration of the vascular wall, smooth muscle cell proliferation, and fibrin deposition within

207 atherosclerotic plaques. [5]. However, this effect is not specific to influenza infection and has

208 also been documented for urinary tract infections [23].

209 The existence of a **specific action** is suggested by the possible antigenic cross-reactivity

210 between influenza components and pro-inflammatory plaque self-antigens [10,30,31].

211 Autoimmune reactions are known to contribute to the progression of atherosclerosis [32,33].

The viral hemagglutinin's binding zone and the apolipoprotein B in LDL particles display significant molecular similarities [34]. The influenza virus might therefore affect lipid metabolism by modulating ongoing immune and inflammatory processes during atherosclerosis. A positive correlation between the respective titers of anti-influenza A immunoglobulin (Ig)G antibodies and anti-oxidized LDL antibodies has been demonstrated in patients with documented progression of atherosclerosis [30]. In the mouse, influenza A virus infection was associated with a loss of high-density lipoprotein anti-inflammatory properties [35], promoting macrophage traffic into arteries [36]. Consequently, the loss of high-density lipoprotein's protective effect might increase LDL oxidation, atherosclerosis progression, and plaque destabilization. Gurevich et al. have hypothesized several possible autoimmune mechanisms: (i) the systemic inflammatory response to infection may enhance lipid peroxidation and the subsequent production of autoantibodies against modified LDL, (ii) direct vessel wall colonization may initiate a local cell-based autoimmune reaction via activation of antigen-presenting cells; and (iii) molecular mimicry may stimulate an atherogenic autoimmune reaction that prompts the development and progression of atherosclerosis.

3   Beneficial effects of influenza vaccination

3.1   *Influenza vaccination prevents cardiovascular events in older adults*

Although it is still difficult to establish a causal relationship between influenza infection and ACS on the basis of epidemiological data, the available evidence has prompted physicians to try a therapeutic approach using IV. Naghavi et *al*. were among the first to hypothesize that IV might protect against incident ACS. In their study population (mean age: 62 years), IV in the influenza season was associated with a reduction in the incidence of ACS during the following

236 influenza season (OR 0.33; 95%CI [0.13-0.82]; *p*=0.017) [37]. Thereafter, many observational

237 studies evidenced a beneficial effect of IV on mortality and on acute ischemic events (**Table**

238 **1**). Although the effectiveness of IV in reducing all-cause mortality in older adults appears to

239 be well established [38–42], its effect on specific cardiovascular mortality [9–13,39,41,42] and

240 the degree of protection are subject to debate. Gross et *al*. estimated that the risk from 27–30%

241 lower in case-control studies and 56–76% lower in cohort studies [43]. In contrast, Udell et al.'s

242 meta-analysis (mean age of the analyzed patients: 67) failed to find a significant effect on

243 cardiovascular mortality [13]. Although the epidemiological data suggest that a beneficial effect

244 may not be present during the summer months [8,10,38,39,44–48] and may not be provided by

245 matched/mismatched vaccine strains in older adults [49], the results of the FLUVACS and

246 FLUCAD early randomized trials consistently evidenced an association between mortality and

247 ischemic events outside the influenza season [10–12].

248 A number of studies have found that IV also protects against major non-fatal cardiovascular

249 events, with a significantly lower risk of hospitalization for heart disease [9,13,38,40], AMI

250 [8,9,13,40,47] and stroke [8,40,50] in older adults. Observational and clinical studies still show

251 discordant results with regard to the protective association with AMI, although the association

252 with stroke is more consistent (**Table 1**). Lastly, repeat vaccination shows a consistent, dose-

253 dependent, protective association with ACS [8,47] and stroke [8,48,50]. Although there are still

254 some gray areas, the studies of repeat vaccination fit with the observational studies and

255 emphasize the need for more robust clinical research on the beneficial effects of IV in older

256 adults.

257

258 *3.2 Cardioprotective effects and possible mechanisms*

As mentioned above, influenza virus can trigger major cardiovascular events and the progression of atherosclerosis (**Figure 1**). Accordingly, the seroprotection provided by IV enables a secondary immune response that neutralizes the virus, prevents host cell colonization, and results in elimination of the pathogen. This widely accepted mechanism is underpinned by a large number of epidemiological, clinical and experimental studies. However, these results do not explain (i) the beneficial effects observed during generally virus-free periods (i.e. the summer months) [8–10,12,38,47], (ii), the sustained post-IV benefits on atheroma plaques in animals that have not been exposed to influenza virus [51], and (iii) the cumulative effect of repeat vaccination [8,39,47,48,50]. Some evidence for a novel, influenza-infection-independent mechanism has emerged.

The influenza-infection-independent mechanism.

Few studies of an influenza-infection-independent mechanism have been performed (**Table 2**). Bermudez-Fajardo et *al*. were the first to study infection-independent effects on atherosclerotic lesions in the ApoE$^{-/-}$ mouse. At high doses, IV modulated the T-cell inflammatory response in fibrolipidic lesions, and was associated with stable plaques, a decrease in the secretion of pro-inflammatory cytokines (interferon gamma, IL-2, and IL-17), and an increase in the secretion of anti-inflammatory cytokines (IL-4) [51]. However, the underlying mechanism could not be determined. A time and dose-dependent increase in anti-influenza IgG1 levels in immunized mice suggesting that IV's protective effect with regard to CVD might be antibody-dependent. The impact on the cytokine response has been assessed by Keshtkar-Jahromi et al. [52]. The pro-inflammatory cytokine tumor necrosis factor-alpha-related weak inducer of apoptosis (TWEAK) is produced by peripheral blood monocytes and contributes to the progression of atherosclerosis. Its activities include a proatherogenic response, the proliferation and migration of smooth muscle cells in atherosclerotic plaques, and increased synthesis of metalloproteinases (thus reducing plaque stability) [53]. In a study of 69 community-dwelling

12

284   older adults, serum levels of TWEAK were found to be significantly and abnormally low four

285   weeks after Fluarix® IV, and the effect was greatest in frail individuals. Furthermore, this

286   decrease was inversely proportional to the vaccine-induced antibody response and was not

287   associated with monocyte/macrophage activation – suggesting that the beneficial effect of IV

288   on circulating TWEAK levels had been induced by the vaccine directly or had been mediated

289   by a monocyte-independent pathway.

290   Using the informational spectrum method, Veljko et al. suggested that vaccine antibodies could

291   act as agonists on atheroprotective pathways. A comparative structural analysis indicated the

292   involvement of the bradykinin B2 receptor (BKB2R), which has a key role in cardiovascular

293   homeostasis [54]. Bradykinin's interaction with its receptor induces an antioxidant and anti-

294   inflammatory response [55], including beneficial coronary dilatation in early-stage ACS and

295   during remodeling after AMI. Furthermore, activation of the BKB2R pathway inhibits

296   apoptosis, inflammation, and myocardial hypertrophy [55]. Lastly, identification of antigenic

297   similarities between viral antigens and oxidized LDL suggested that a cross-reaction was

298   possible [34]. One can hypothesize that the antibodies elicited by IV bind to oxidized LDL and

299   prevent it from being internalized by macrophages – thus limiting the development and

300   progression of atherosclerosis. However, this mechanism is has yet to be demonstrated in

301   practice, and further research is warranted.

302

303   *3.3   Influenza vaccination and infection: paradoxical responses*

304   It has been suggested that IV can serve as an *in vivo* model of the mild inflammatory stimulation

305   [76]. Influenza vaccination causes transient changes in several biomarkers of inflammation and

306   lipid status. In healthy older adults, the serum CRP level increases slightly but significantly 1

307   to 3 days after IV [56]. In patients with carotid stenosis, the CRP level after IV was 1.3 times

308 higher than in healthy controls (95% CI, 0.84–2.02, P=0.240) [57]. Increased serum acute-

309 phase protein levels have been linked to atherosclerotic plaque instability and ACS. In a meta-

310 analysis of more than 7000 patients with coronary events, subjects with a serum CRP level in

311 the upper tertile had a higher risk of acute cardiovascular events (combined OR 1.49, 95% CI,

312 1.37 to 1.62; χ^2=10.6, P=0.01) [58].

313 If exaggerated inflammation after an influenza infection exacerbates atherosclerotic lesions,

314 why does IV have the opposite effect? And why does influenza infection not confer the same

315 long-term protection as IV?

316 We suggest that these apparently paradoxical observations can be explained by the concept of

317 hormesis, whereby a low dose of a stressor initiates compensatory biological processes,

318 activates adaptive systems, and confers protection against subsequent exposure to a high dose

319 of the same stressor (**Figure 2**). This phenomenon is also referred to as ischemic

320 preconditioning and is widely recognized in the field of CVD. In an animal study, the

321 myocardial infarction size was 75% smaller after four prior 5-minute periods of ischemia

322 separated by 5 minutes reperfusion periods [59]. Thus, a "sub-damaging" stimulus confers

323 resistance to ischemic heart injury. Influenza vaccination induces a mild, transient systemic

324 inflammatory response, which may well explain why this vaccination is not associated with an

325 increased risk of ACS. The results of clinical and animal studies suggest that hormesis can

326 protect against many aging-related diseases, including diabetes, CVD, cancers and

327 neurocognitive disorders [60].

328 Furthermore, a wide variety of stimuli (such as thermal stress [61], bradykinin, and nitric oxide

329 (NO) [60]) can trigger the protective signal. As mentioned above, Veljko et *al.* highlighted

330 similarities between an anti-hemagglutinin A1 antibody and bradykinin B2, with the potential

331 for agonism on the BKB2R [55]. At the endothelial level, activated BKB2R induces NO release

332 and thus the generation of reactive oxygen species - the key factor in oxidative stress. Reactive

oxygen species have a hormetic effect *in vitro*; low-level, transient oxidative stress reduces acute inflammatory responses by cultured endothelial cells [62]. Furthermore, the direct administration of reactive oxygen species at concentrations close to those observed during myocardial reperfusion causes myocardial injury [63]. Influenza vaccination is associated with a slight increase in exhaled NO [64]. We therefore suggest that IV promotes ischemic preconditioning, which in turn accounts for (at least in part) the protective effects in atherosclerotic plaques.

4   Discussion

The mortality rates associated with CHD and influenza infection are elevated in older adults. Population aging and demographic changes are prompting the need for effective means of preventing both pathologies. There is solid evidence for a correlation between IV and a reduction in cardiovascular events – suggesting that IV is a high-potential strategy for protecting older adults. Although these findings must be considered with caution, the present review raises several hypotheses for further investigation.

Aging and influenza vaccination

Aging is accompanied by immunosenescence, increased susceptibility to infection, and reduced responses to vaccination. Studies of geriatric populations have largely identified the age over 65 as a criterion for defining older persons. In the field of geriatric medicine, it is widely accepted that an aging phenotype is a better criterion for physiologic age than chronological age *per se*. The prevalence of frailty (a geriatric syndrome marked by loss of function and physiological reserve) rises with age. It has been suggested that frailty is a causative and prognostic factor for CVD. In a meta-analysis of 54,250 older adults, CVD was associated with

15

357 an OR of between 2.7 and 4.1 for prevalent frailty and of 1.5 for incident frailty in those who

358 were not frail at baseline [65]. Regardless of age, the severity of the underlying disease,

359 comorbidities, and disability, frailty is a powerful predictor of mortality in patients with CVD.

360 In older adults with severe CHD or heart failure, the prevalence of frailty ranged from 50% to

361 54%, and was associated with an increased risk of all-cause mortality (OR from 1.6 to 4.0) [65].

362 As frailty is an independent risk factor for CVD in older adults, it will be important to consider

363 this variable in future studies of vaccine efficacy in older subjects and is of crucial importance

364 in understanding the cardiovascular health benefits of IV.

365

Vitamin D: a potential adjuvant?

367 Vitamin D is an important factor in cardiovascular health. In epidemiological studies, vitamin

368 D deficiency is consistently associated with an higher risk of CVD [66]. *In vitro* studies have

369 shown that vitamin D suppresses inflammation, reduces the internalization of ox-LDL,

370 decreases the expression of many pro-inflammatory cytokines, and polarizes T cells towards a

371 Th2 response [74,76]. However, RCT and meta-analysis findings regarding the beneficial

372 effects of vitamin D supplementation (standard dose, high dose and/or their association with

373 calcium supplementation) on CVD outcomes have remained largely disappointing. [67,68].

374 Two of the factors limiting assessment of the effect of vitamin D are baseline status and efficacy

375 of supplementation. The definition of hypovitaminosis D and the "optimal" status of vitamin D

376 is still controversial in vitamin D research [69]. In addition, the administration of 25 (OH) D is

377 often only based on tests that still need to be standardized to allow for the pooling of research

378 data [70].

379   Vitamin D deficiency affects nearly 50% of older adults in Europe, and its prevalence increases

380   with age [71]. The association between low vitamin D levels and frailty has been evaluated in

381   several studies. A recent systematic review with meta-analysis found that people with low

382   serum levels of 25 (OH) D had a higher risk of frailty [72]. This association may be explained

383   by the effect of vitamin D on bone health and muscle strength.

384   Vitamin D evaluated in the context of the IV did not find a correlation between 25-(OH) D

385   levels and response to the vaccine. In a recent clinical trial, vitamin D supplementation in older

386   adults who were deficient at baseline (<30 ng/mL) and then vaccinated was not associated with

387   a difference in the antibody response but interestingly, was associated with lymphocyte

388   polarization towards a Th2 tolerogenic immune response, supporting the immunomodulatory

389   effect of vitamin D [73].

390   The link between vitamin D deficiency, cardiovascular disease, and frailty reinforced the need

391   to define geriatric populations in a different way than age and to consider vitamin D status in

392   the assessment of CVD in future studies targeting older people. Moreover, future studies should

393   help to determine whether vitamin D supplementation associated with IV prevents CVD

394   outcomes in older adults with different basal serum 25-(OH) D levels.

395

396   **5   Research perspectives**

397   The results reviewed above emphasize the need for further research on IV and notably its

398   potential value in preventive medicine and its benefits in older adults **(Figure 3).**

399

400   *5.1   The intensification of IV clinical research*

401 The fact that meta-analyses of IV and its cardiovascular effects continue to produce

402 contradictory results emphasizes the need for adequately powered, prospective, randomized

403 trials. In fact, two large ongoing trials are evaluating the effect of IV on cardiovascular

404 outcomes. The IAMI trial randomized 4400 patients with ST-elevation myocardial infarction

405 or non-ST-elevation myocardial infarction undergoing coronary angiography, and will evaluate

406 the effect of IV on death and cardiovascular outcomes at 1 year [74]. The IVVE 3-year follow-

407 up trial will probe a possible reduction in the occurrence of adverse cardiovascular events in

408 patients with heart failure [75]. These two clinical trials will hopefully provide the solid data

409 needed to define the effect of IV on cardiovascular risk.

410

411 *5.2   Influenza vaccination: an immune modulation strategy in atherosclerosis*

412 The possibility of developing immune therapies to reduce cardiovascular risk has led current

413 research into the field of vaccination against atherosclerosis [76]. The demonstration of

414 immunomodulation by IV is therefore a promising direction in the area of atherosclerosis. The

415 evidence of a similar effect in other pathologies underpinned by a similar inflammatory process

416 seemed to be a simple first approach to support this immunomodulatory effect of the vaccine.

417 In this perspective, we examined the question of the effect of the vaccine on other age-related

418 diseases, which have an inflammatory component in common with atherosclerosis.

419 Aging phenotype is characterized by an *immunosenescence*, responsible for reducing

420 adaptability to various stresses, and a concomitant increase in pro-inflammatory status or

421 "*inflammaging*". It is well established that this pro-inflammatory condition is a common

422 pathophysiological mechanism and a major risk factor for aging-related diseases, including

423 atherosclerosis **(Figure 4) [77–79]**.

424 This pathogenesis common to age-related diseases leads us to consider a pleiotropic effect of

425 the vaccine as additional evidence of an immunomodulatory effect. To the best of our

426 knowledge, only a few studies have examined the effect of IV on other aging-related diseases.

427 Prior exposure to diphtheria, tetanus and polio vaccines (but not influenza vaccine) was

428 associated with a lower risk of Alzheimer's disease in older adults (OR [95%CI] = 0.41, [0.27–

429 0.62], 0.60 [0.37–0.99], and 0.75 [0.54–1.04], respectively) [80]. Furthermore, IV was

430 associated with significantly lower major-cause mortality for stroke (hazard ratio (HR)=0.35),

431 renal disease (HR=0.40), diabetes mellitus (HR=0.45), pneumonia (HR=0.47), and cancer

432 (HR=0.74) [41]. Selectively stimulating the atheroprotective immune response while inhibiting

433 pathogenic immune responses following influenza vaccination offers new and interesting

434 therapeutic possibilities.

435 Second, few studies have focused on the specific mechanistic processes that underlie IV (**Table**

436 **2**). The heterogeneity of these studies' approaches and designs weakens their coherence and

437 validity, and prevents firm conclusions from being drawn. Possible antigenic mimicry between

438 influenza A hemagglutinin (HA) and apolipoprotein B-100 (ApoB-100) [30,34] is a promising

439 putative mechanism. Both native and aldehyde-modified ApoB-100 are important targets for

440 protective responses in atherosclerosis. Anti-apoB-100 antibodies induced a 40 to 70% decrease

441 in atherosclerosis and inflammation in animal models [81]. A transient anti-inflammatory Th2

442 response (mediated by anti-ApoB-100 IgG1) was associated with a relative decrease in the size

443 of atherosclerotic lesions in the ApoE $^{-/-}$ mouse, as is also observed after IV. We suggest that the

444 ApoB-100 peptide might be a valuable tool for understanding cellular processes after

445 vaccination.

446 Scientific interest in IV and the possibly associated cardiovascular protection continues to grow.

447 Although clinical data are gradually strengthening this association, there is a marked lack of

448 molecular immunology studies in this field. The generation of additional biological data will

449 justify greater efforts for defining IV as an atheroprotective measure and for expanding current

450 treatment options. Confirmation of IV's cardioprotective effect and characterization of the

451 underlying mechanism might lead to greater vaccination coverage and synergistic preventive

452 effects on influenza infection and CVD.

453

454 *5.3 Development of a more effective influenza vaccine*

455 There is a great need for vaccines that optimally stimulate the immune system of older adults.

456 A meta-analysis of more than 30 vaccine studies showed that the clinical effectiveness ranged

457 from 70 to 90% in young adults, and from 17 to 53% in older adults [82]. To increase

458 effectiveness, several optimized vaccination strategies have been developed; these include

459 intradermal vaccines, high-dose vaccines, and the adjuvant oil-in-water emulsion vaccine

460 MF59. In older adults, all these formulations are slightly more immunogenic than standard

461 trivalent inactivated vaccines. In a meta-analysis of studies in older adults, a high-dose vaccine

462 was significantly more effective (relative vaccine efficacy, 17.7%; 95% CI, 6.6–27.4%) in

463 reducing major cardiorespiratory events, relative to a standard dose vaccine [83]. However, the

464 results were discordant in subgroup analyses of hospitalizations due to ACS and strokes.

465 Similarly, the MF59 adjuvanted vaccine was associated with a significant reduction in

466 hospitalizations for ACS and stroke (adjusted OR 0.13; 95% CI 0.03–0.65 and adjusted OR

467 0.07; 95% CI 0.01–0.48 respectively) during viral circulation, relative to non-vaccinated

468 patients [84]. The establishment of new vaccination protocols is needed to prevent the incidence

469 of infectious diseases and reduce the associated mortality. In this perspective, Vardeny et *al.*

470 are conducting a randomized controlled trial (the INVESTED trial) of a high-dose vaccine and

471 the standard vaccine; a lower incidence of cardiovascular adverse events with the high-dose

472 vaccine would suggest a dose-response relationship between IV and cardiovascular prevention

473 [85].

474

475 *5.4 Influenza vaccine: an effective, low-cost treatment for CVD?*

476 Interventions aimed at preventing CVD (including lifestyle interventions, risk factor control,

477 and medications such as beta-blockers, aspirin/clopidogrel, angiotensin II receptor blockers,

478 and statins) are as effective in older adults as they are in younger adults. However, these

479 strategies are under-used in older adults [86,87]. Effective intervention is complicated by

480 ageism, comorbidities, and limited access to age-appropriate care. Adverse drug reactions

481 (although often preventable) are frequent. Cardiovascular medications were the medications

482 most frequently implicated (26.0% of cases) in adverse reactions in older adults [88].

483 Recognition of the beneficial effect of IV offers a new therapeutic opportunity for preventing

484 CVD. Vaccination is a well-tolerated, inexpensive, effective method for reducing morbidity in

485 patients at high risk of CVD. Vaccination has a favorable cost/benefit ratio, when compared

486 with current pharmacological treatments [26]. A paradigm shift may be necessary to encourage

487 clinicians to see IV as a cost-effective, safe, adjunct prevention strategy for patients with CVD.

488

489 **Conclusion**

490 Cardiovascular disease and influenza infection are leading causes of death worldwide, and the

491 disease burdens are greater in people over the age 65. There is growing evidence of a link

492 between IV and a reduction in the incidence of cardiovascular events. This is firstly based on

493 prevention of the cardiovascular complications of influenza infection. We also suggest that IV

494 has other infection-independent cardioprotective effects. The scientific interest in IV and its

495 cardiovascular protection is and continues to grow. However, its specific effect remains largely

496 controversial and difficult to share despite promising results. The provision of additional

497 biological data will justify a greater investment in order to define IV as an atheroprotective

498 vaccine.

499

500 **Authors' contributions**

501 AA and BF made substantial contributions to study conception and design. AA wrote

502 the manuscript. AA, JM, DA, CD, GG and BF have made substantial contributions to the final

503 manuscript. All authors read and approved the final manuscript.

References

[1]   WHO. Projections of mortality and causes of death, 2016 to 2060. WHO 2018. http://www.who.int/healthinfo/global_burden_disease/projections/en/ (accessed May 3, 2019).

[2]   WHO. Global Influenza Strategy for 2019-2030. WHO 2019. http://www.who.int/influenza/global_influenza_strategy_2019_2030/en/ (accessed April 20, 2019).

[3]   Madjid M, Miller CC, Zarubaev VV, Marinich IG, Kiselev OI, Lobzin YV, et al. Influenza epidemics and acute respiratory disease activity are associated with a surge in autopsy-confirmed coronary heart disease death: results from 8 years of autopsies in 34 892 subjects. Eur Heart J 2007;28:1205–10. https://doi.org/10.1093/eurheartj/ehm035.

[4]   Haidari M, Wyde PR, Litovsky S, Vela D, Ali M, Casscells SW, et al. Influenza virus directly infects, inflames, and resides in the arteries of atherosclerotic and normal mice. Atherosclerosis 2010;208:90–6. https://doi.org/10.1016/j.atherosclerosis.2009.07.028.

[5]   Naghavi M. Influenza Infection Exerts Prominent Inflammatory and Thrombotic Effects on the Atherosclerotic Plaques of Apolipoprotein E-Deficient Mice. Circulation 2003;107:762–8. https://doi.org/10.1161/01.CIR.0000048190.68071.2B.

[6]   Suo J, Zhao L, Wang J, Zhu Z, Zhang H, Gao R. Influenza virus aggravates the ox-LDL-induced apoptosis of human endothelial cells via promoting p53 signaling: Influenza Virus Aggravates the Ox-LDL-Induced Apoptosis. J Med Virol 2015;87:1113–23. https://doi.org/10.1002/jmv.24166.

[7]   Visseren FLJ, Bouwman JJM, Bouter KP, Diepersloot RJA, de Groot PG, Erkelens DW. Procoagulant Activity of Endothelial Cells after Infection with Respiratory Viruses. Thromb Haemost 2000;84:319–24. https://doi.org/10.1055/s-0037-1614014.

[8]   Chiang M-H, Wu H-H, Shih C-J, Chen Y-T, Kuo S-C, Chen T-L. Association between influenza vaccination and reduced risks of major adverse cardiovascular events in elderly patients. Am Heart J 2017;193:1–7. https://doi.org/10.1016/j.ahj.2017.07.020.

[9]   Phrommintikul A, Kuanprasert S, Wongcharoen W, Kanjanavanit R, Chaiwarith R, Sukonthasarn A. Influenza vaccination reduces cardiovascular events in patients with acute coronary syndrome. Eur Heart J 2011;32:1730–5. https://doi.org/10.1093/eurheartj/ehr004.

[10]   Ciszewski A, Bilinska ZT, Brydak LB, Kepka C, Kruk M, Romanowska M, et al. Influenza vaccination in secondary prevention from coronary ischaemic events in coronary artery disease: FLUCAD study. Eur Heart J 2008;29:1350–8. https://doi.org/10.1093/eurheartj/ehm581.

[11]   Gurfinkel EP, de la Fuente RL, Mendiz O, Mautner B. Influenza Vaccine Pilot Study in Acute Coronary Syndromes and Planned Percutaneous Coronary Interventions: The FLU Vaccination Acute Coronary Syndromes (FLUVACS) Study. Circulation 2002;105:2143–7. https://doi.org/10.1161/01.CIR.0000016182.85461.F4.

[12]   Gurfinkel EP, Leon de la Fuente R, Mendiz O, Mautner B. Flu vaccination in acute coronary syndromes and planned percutaneous coronary interventions (FLUVACS) Study: One-year follow-up. Eur Heart J 2004;25:25–31. https://doi.org/10.1016/j.ehj.2003.10.018.

[13]   Udell JA, Zawi R, Bhatt DL, Keshtkar-Jahromi M, Gaughran F, Phrommintikul A, et al. Association Between Influenza Vaccination and Cardiovascular Outcomes in High-Risk Patients: A Meta-analysis. JAMA 2013;310:1711–20. https://doi.org/10.1001/jama.2013.279206.

[14]   Hansson GK. Inflammation, Atherosclerosis, and Coronary Artery Disease. N Engl J Med 2005;352:1685–95.

[15]   Ross R. Atherosclerosis -- An Inflammatory Disease. N Engl J Med 1999;340:115–26. https://doi.org/10.1056 / NEJM199901143400207.

[16]   Libby Peter, Ridker Paul M., Maseri Attilio. Inflammation and Atherosclerosis. Circulation 2002;105:1135–43. https://doi.org/10.1161/hc0902.104353.

[17]   Wang Julie C., Bennett Martin. Aging and Atherosclerosis. Circ Res 2012;111:245–59. https://doi.org/10.1161/CIRCRESAHA.111.261388.

[18] Katsuumi G, Shimizu I, Yoshida Y, Minamino T. Vascular Senescence in Cardiovascular and Metabolic Diseases. Front Cardiovasc Med 2018;5:18. https://doi.org/10.3389/fcvm.2018.00018.

[19] Gerhard-Herman M, Smoot LB, Wake N, Kieran MW, Kleinman ME, Miller DT, et al. Mechanisms of premature vascular aging in children with Hutchinson-Gilford Progeria syndrome. Hypertension 2012;59:92–7. https://doi.org/10.1161/HYPERTENSIONAHA.111.180919.

[20] Fabricant CG. Virus-induced atherosclerosis. J Exp Med 1978;148:335–40. https://doi.org/10.1084/jem.148.1.335.

[21] Tufano A, Di Capua M, Coppola A, Conca P, Cimino E, Cerbone A, et al. The Infectious Burden in Atherothrombosis. Semin Thromb Hemost 2012;38:515–23. https://doi.org/10.1055/s-0032-1315759.

[22] Collins SD. Excess Mortality from Causes Other than Influenza and Pneumonia during Influenza Epidemics. Public Health Rep 1896-1970 1932;47:2159. https://doi.org/10.2307/4580606.

[23] Smeeth L, Hubbard R. Risk of Myocardial Infarction and Stroke after Acute Infection or Vaccination. N Engl J Med 2004;351:2611–8.

[24] Nguyen JL, Yang W, Ito K, Matte TD, Shaman J, Kinney PL. Seasonal Influenza Infections and Cardiovascular Disease Mortality. JAMA Cardiol 2016;1:274–81. https://doi.org/10.1001/jamacardio.2016.0433.

[25] Guan X-R, Li X, Xin X-M, Jiang L-X, Cui L-Y, Wang L-F, et al. Influenza Virus Infection and Risk of Acute Myocardial Infarction. Inflammation 2008;31:266–72. https://doi.org/10.1007/s10753-008-9074-2.

[26] MacIntyre CR, Heywood AE, Kovoor P, Ridda I, Seale H, Tan T, et al. Ischaemic heart disease, influenza and influenza vaccination: a prospective case control study. Heart 2013;99:1843–8. https://doi.org/10.1136/heartjnl-2013-304320.

[27] Kwong JC, Schwartz KL, Campitelli MA, Chung H, Crowcroft NS, Karnauchow T, et al. Acute Myocardial Infarction after Laboratory-Confirmed Influenza Infection. N Engl J Med 2018;378:345–53. https://doi.org/10.1056/NEJMoa1702090.

[28] Wang S, Quang Le T, Chida J, Cisse Y, Yano M, Kido H. Mechanisms of matrix metalloproteinase-9 upregulation and tissue destruction in various organs in influenza A virus infection. J Med Invest 2010;57:26–34. https://doi.org/10.2152/jmi.57.26.

[29] Corrales-Medina VF, Madjid M, Musher DM. Role of acute infection in triggering acute coronary syndromes. Lancet Infect Dis 2010;10:83–92. https://doi.org/10.1016/S1473-3099(09)70331-7.

[30] Gurevich VS. Influenza, autoimmunity and atherogenesis. Autoimmun Rev 2005;4:101–5. https://doi.org/10.1016/j.autrev.2004.10.006.

[31] Madjid M. Influenza and Cardiovascular Disease: A New Opportunity for Prevention and the Need for Further Studies. Circulation 2003;108:2730–6. https://doi.org/10.1161/01.CIR.0000102380.47012.92.

[32] Carbone F, Nencioni A, Mach F, Vuilleumier N, Montecucco F. Evidence on the pathogenic role of auto-antibodies in acute cardiovascular diseases. Thromb Haemost 2013;109:854–68. https://doi.org/10.1160/TH12-10-0768.

[33] Vuilleumier NS and N. Auto-Antibodies As Possible Markers and Mediators of Ischemic, Dilated, and Rhythmic Cardiopathies_. Curr Drug Targets 2015. http://www.eurekaselect.com/126305/article (accessed September 12, 2019).

[34] Pleskov VM, Bannikov AI, Zaĭtsev IV. The receptor-mediated endocytosis of influenza viruses and low-density lipoproteins by tissue cells. Vopr Virusol 1994;39:121–5.

[35] Van Lenten BJ, Wagner AC, Nayak DP, Hama S, Navab M, Fogelman AM. High-Density Lipoprotein Loses Its Anti-Inflammatory Properties During Acute Influenza A Infection. Circulation 2001;103:2283–8. https://doi.org/10.1161/01.CIR.103.18.2283.

[36] Van Lenten BJ. Influenza Infection Promotes Macrophage Traffic Into Arteries of Mice That Is Prevented by D-4F, an Apolipoprotein A-I Mimetic Peptide. Circulation 2002;106:1127–32. https://doi.org/10.1161/01.CIR.0000030182.35880.3E.

[37] Naghavi M, Barlas Z, Siadaty S, Naguib S, Madjid M, Casscells W. Association of Influenza Vaccination and Reduced Risk of Recurrent Myocardial Infarction. Circulation 2000;102:3039–45. https://doi.org/10.1161/01.CIR.102.25.3039.

[38]   Liu I-F, Huang C-C, Chan W-L, Huang P-H, Chung C-M, Lin S-J, et al. Effects of annual influenza vaccination on mortality and hospitalization in elderly patients with ischemic heart disease: A nationwide population-based study. Prev Med 2012;54:431–3. https://doi.org/10.1016/j.ypmed.2012.03.020.

[39]   Voordouw ACG, Sturkenboom MCJM, Dieleman JP, Stijnen T, Smith DJ, Lei J van der, et al. Annual Revaccination Against Influenza and Mortality Risk in Community-Dwelling Elderly Persons. JAMA 2004;292:2089–95. https://doi.org/10.1001/jama.292.17.2089.

[40]   Nichol KL, Nordin J, Mullooly J, Lask R, Fillbrandt K, Iwane M. Influenza Vaccination and Reduction in Hospitalizations for Cardiac Disease and Stroke among the Elderly. N Engl J Med 2003;348:1322–32. https://doi.org/10.1056/NEJMoa025028.

[41]   Wang C-S, Wang S-T, Lai C-T, Lin L-J, Chou P. Impact of influenza vaccination on major cause-specific mortality. Vaccine 2007;25:1196–203. https://doi.org/10.1016/j.vaccine.2006.10.015.

[42]   Armstrong BG, Mangtani P, Fletcher A, Kovats S, McMichael A, Pattenden S, et al. Effect of influenza vaccination on excess deaths occurring during periods of high circulation of influenza: cohort study in elderly people. BMJ 2004;329:660. https://doi.org/10.1136/bmj.38198.594109.AE.

[43]   Gross PA. The Efficacy of Influenza Vaccine in Elderly Persons: A Meta-Analysis and Review of the Literature. Ann Intern Med 1995;123:518–27. https://doi.org/10.7326/0003-4819-123-7-199510010-00008.

[44]   Vamos EP, Pape UJ, Curcin V, Harris MJ, Valabhji J, Majeed A, et al. Effectiveness of the influenza vaccine in preventing admission to hospital and death in people with type 2 diabetes. Can Med Assoc J 2016;188:E342–51. https://doi.org/10.1503/cmaj.151059.

[45]   Heffelfinger JD, Heckbert SR, Psaty BM, Weiss NS, Thompson WW, Bridges CB, et al. Influenza Vaccination and Risk of Incident Myocardial Infarction. Hum Vaccin 2006;2:161–6. https://doi.org/10.4161/hv.2.4.2943.

[46]   Grau Armin J., Fischer Beate, Barth Cordula, Ling Paul, Lichy Christoph, Buggle Florian. Influenza Vaccination Is Associated With a Reduced Risk of Stroke. Stroke 2005;36:1501–6. https://doi.org/10.1161/01.STR.0000170674.45136.80.

[47]   Chen C-I, Kao P-F, Wu M-Y, Fang Y-A, Miser JS, Liu J-C, et al. Influenza Vaccination is Associated with Lower Risk of Acute Coronary Syndrome in Elderly Patients with Chronic Kidney Disease. Medicine (Baltimore) 2016;95. https://doi.org/10.1097/MD.0000000000002588.

[48]   Lin H-C, Chiu H-F, Ho S-C, Yang C-Y. Association of Influenza Vaccination and Reduced Risk of Stroke Hospitalization among the Elderly: A Population-Based Case-Control Study. Int J Environ Res Public Health 2014;11:3639–49. https://doi.org/10.3390/ijerph110403639.

[49]   Hsu S-Y, Chen F-L, Liaw Y-P, Huang J-Y, Nfor ON, Chao D-Y. A Matched Influenza Vaccine Strain Was Effective in Reducing the Risk of Acute Myocardial Infarction in Elderly Persons: A Population-Based Study. Medicine (Baltimore) 2016;95. https://doi.org/10.1097/MD.0000000000002869.

[50]   Lavallée P, Perchaud V, Gautier-Bertrand M, Grabli D, Amarenco P. Association Between Influenza Vaccination and Reduced Risk of Brain Infarction. Stroke 2002;33:513–8. https://doi.org/10.1161/hs0202.102328.

[51]   Bermúdez-Fajardo A, Oviedo-Orta E. Influenza vaccination promotes stable atherosclerotic plaques in apoE knockout mice. Atherosclerosis 2011;217:97–105. https://doi.org/10.1016/j.atherosclerosis.2011.03.019.

[52]   Keshtkar-Jahromi M, Ouyang M, Keshtkarjahromi M, Almed S, Li H, Walston JD, et al. Effect of influenza vaccine on tumor necrosis factor-like weak inducer of apoptosis (TWEAK) in older adults. Vaccine 2018;36:2220–5. https://doi.org/10.1016/j.vaccine.2017.11.045.

[53]   Blanco-Colio L M. TWEAK and Fn14. New players in the pathogenesis of atherosclerosis. Front Biosci 2007;12:3648–55. https://doi.org/10.2741/2341.

[54]   Veljkovic V, Glisic S, Veljkovic N, Bojic T, Dietrich U, Perovic VR, et al. Influenza vaccine as prevention for cardiovascular diseases: Possible molecular mechanism. Vaccine 2014;32:6569–75. https://doi.org/10.1016/j.vaccine.2014.07.007.

[55]   Chao J, Chao L. Kallikrein-kinin in stroke, cardiovascular and renal disease: Kallikrein in cardiovascular and renal disease. Exp Physiol 2005;90:291–8. https://doi.org/10.1113/expphysiol.2004.028464.

[56]   Posthouwer D, Voorbij HAM, Grobbee DE, Numans ME, van der Bom JG. Influenza and pneumococcal vaccination as a model to assess C-reactive protein response to mild inflammation. Vaccine 2004;23:362–5. https://doi.org/10.1016/j.vaccine.2004.05.035.

[57] Carty Cara L., Heagerty Patrick, Nakayama Karen, McClung E. Clair, Lewis Julieann, Lum Dawn, et al. Inflammatory Response After Influenza Vaccination in Men With and Without Carotid Artery Disease. Arterioscler Thromb Vasc Biol 2006;26:2738–44. https://doi.org/10.1161/01.ATV.0000248534.30057.b5.

[58] Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med 2004;350:1387–97. https://doi.org/10.1056/NEJMoa032804.

[59] Murry C, Jennings R B, Reimer K A. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986;74:1124–36. https://doi.org/10.1161/01.CIR.74.5.1124.

[60] Mattson MP. Hormesis et résistance aux maladies: activation des voies de réponse au stress cellulaire. Hum Exp Toxicol 2008;27:155–62. https://doi.org/10.1177/0960327107083417.

[61] Ferrera R, Bopassa J-C, Angoulvant D, Ovize M. Post-conditioning protects from cardioplegia and cold ischemia via inhibition of mitochondrial permeability transition pore. J Heart Lung Transplant 2007;26:604–9. https://doi.org/10.1016/j.healun.2007.02.009.

[62] Zahler S, Kupatt C, Becker BF. Endothelial preconditioning by transient oxidative stress reduces inflammatory responses of cultured endothelial cells to TNF-α. FASEB J 2000;14:555–64. https://doi.org/10.1096/fasebj.14.3.555.

[63] Vinten-Johansen J. Postconditioning: a mechanical maneuver that triggers biological and molecular cardioprotective responses to reperfusion. Heart Fail Rev 2007;12:235–44. https://doi.org/10.1007/s10741-007-9024-3.

[64] Thomas PS, Ng C, Elsing M, Yates DH. Influenza vaccination: Changes in exhaled nitric oxide levels and sputum cytology. Respirology 1999;4:355–8. https://doi.org/10.1046/j.1440-1843.1999.00204.x.

[65] Afilalo J, Karunananthan S, Eisenberg MJ, Alexander KP, Bergman H. Role of Frailty in Patients With Cardiovascular Disease. Am J Cardiol 2009;103:1616–21. https://doi.org/10.1016/j.amjcard.2009.01.375.

[66] Wang TJ. Vitamin D and Cardiovascular Disease. Annu Rev Med 2016;67:261–72. https://doi.org/10.1146/annurev-med-051214-025146.

[67] Barbarawi M, Kheiri B, Zayed Y, Barbarawi O, Dhillon H, Swaid B, et al. Vitamin D Supplementation and Cardiovascular Disease Risks in More Than 83 000 Individuals in 21 Randomized Clinical Trials: A Meta-analysis. JAMA Cardiol 2019. https://doi.org/10.1001/jamacardio.2019.1870.

[68] Manson JE, Cook NR, Lee I-M, Christen W, Bassuk SS, Mora S, et al. Vitamin D Supplements and Prevention of Cancer and Cardiovascular Disease. N Engl J Med 2019;380:33–44. https://doi.org/10.1056/NEJMoa1809944.

[69] Sempos CT, Vesper HW, Phinney KW, Thienpont LM, Coates PM, Vitamin D Standardization Program (VDSP). Vitamin D status as an international issue: national surveys and the problem of standardization. Scand J Clin Lab Investig Suppl 2012;243:32–40. https://doi.org/10.3109/00365513.2012.681935.

[70] Sempos CT, Heijboer AC, Bikle DD, Bollerslev J, Bouillon R, Brannon PM, et al. Vitamin D assays and the definition of hypovitaminosis D: results from the First International Conference on Controversies in Vitamin D. Br J Clin Pharmacol 2018;84:2194–207. https://doi.org/10.1111/bcp.13652.

[71] Hilger J, Friedel A, Herr R, Rausch T, Roos F, Wahl DA, et al. A systematic review of vitamin D status in populations worldwide. Br J Nutr 2014;111:23–45. https://doi.org/10.1017/S0007114513001840.

[72] Zhou J, Huang P, Liu P, Hao Q, Chen S, Dong B, et al. Association of vitamin D deficiency and frailty: A systematic review and meta-analysis. Maturitas 2016;94:70–6. https://doi.org/10.1016/j.maturitas.2016.09.003.

[73] Goncalves-Mendes N, Talvas J, Dualé C, Guttmann A, Corbin V, Marceau G, et al. Impact of Vitamin D Supplementation on Influenza Vaccine Response and Immune Functions in Deficient Elderly Persons: A Randomized Placebo-Controlled Trial. Front Immunol 2019;10. https://doi.org/10.3389/fimmu.2019.00065.

[74] Fröbert O, Götberg M, Angerås O, Jonasson L, Erlinge D, Engström T, et al. Design and rationale for the Influenza vaccination After Myocardial Infarction (IAMI) trial. A registry-based randomized clinical trial. Am Heart J 2017;189:94–102. https://doi.org/10.1016/j.ahj.2017.04.003.

[75] Loeb M, Dokainish H, Dans A, Palileo-Villanueva LM, Roy A, Karaye K, et al. Randomized controlled trial of influenza vaccine in patients with heart failure to reduce adverse vascular events (IVVE): Rationale and design. Am Heart J 2019;212:36–44. https://doi.org/10.1016/j.ahj.2019.02.009.

[76] Nilsson Jan, Hansson Göran K. Vaccination Strategies and Immune Modulation of Atherosclerosis. Circ Res 2020;126:1281–96. https://doi.org/10.1161/CIRCRESAHA.120.315942.

[77] Franceschi C, Bonafè M, Valensin S, Olivieri F, Luca MD, Ottaviani E, et al. Inflamm-aging: An Evolutionary Perspective on Immunosenescence. Ann N Y Acad Sci 2000;908:244–54. https://doi.org/10.1111/j.1749-6632.2000.tb06651.x.

[78] Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A. Inflammaging: a new immune–metabolic viewpoint for age-related diseases. Nat Rev Endocrinol 2018;14:576–90. https://doi.org/10.1038/s41574-018-0059-4.

[79] Ferrucci L, Fabbri E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. Nat Rev Cardiol 2018;15:505. https://doi.org/10.1038/s41569-018-0064-2.

[80] Verreault R, Laurin D, Lindsay J, De Serres G. Past exposure to vaccines and subsequent risk of Alzheimer's disease. CMAJ 2001;165:1495–8.

[81] Asciutto G, Wigren M, Fredrikson GN, Mattisson IY, Grönberg C, Alm R, et al. Apolipoprotein B-100 Antibody Interaction With Atherosclerotic Plaque Inflammation and Repair Processes. Stroke 2016;47:1140–3. https://doi.org/10.1161/STROKEAHA.116.012677.

[82] Goodwin K, Viboud C, Simonsen L. Antibody response to influenza vaccination in the elderly: A quantitative review. Vaccine 2006;24:1159–69. https://doi.org/10.1016/j.vaccine.2005.08.105.

[83] DiazGranados CA, Robertson CA, Talbot HK, Landolfi V, Dunning AJ, Greenberg DP. Prevention of serious events in adults 65 years of age or older: A comparison between high-dose and standard-dose inactivated influenza vaccines. Vaccine 2015;33:4988–93. https://doi.org/10.1016/j.vaccine.2015.07.006.

[84] Puig-Barberà J, Díez-Domingo J, Varea ÁB, Chavarri GS, Rodrigo JAL, Hoyos SP, et al. Effectiveness of MF59™-adjuvanted subunit influenza vaccine in preventing hospitalisations for cardiovascular disease, cerebrovascular disease and pneumonia in the elderly. Vaccine 2007;25:7313–21. https://doi.org/10.1016/j.vaccine.2007.08.039.

[85] Vardeny O, Udell JA, Joseph J, Farkouh ME, Hernandez AF, McGeer AJ, et al. High-dose influenza vaccine to reduce clinical outcomes in high-risk cardiovascular patients: Rationale and design of the INVESTED trial. Am Heart J 2018;202:97–103. https://doi.org/10.1016/j.ahj.2018.05.007.

[86] Meid AD, Quinzler R, Freigofas J, Saum K-U, Schöttker B, Holleczek B, et al. Medication Underuse in Aging Outpatients with Cardiovascular Disease: Prevalence, Determinants, and Outcomes in a Prospective Cohort Study. PloS One 2015;10:e0136339. https://doi.org/10.1371/journal.pone.0136339.

[87] Fleg JL, Aronow WS, Frishman WH. Cardiovascular drug therapy in the elderly: benefits and challenges. Nat Rev Cardiol 2011;8:13–28. https://doi.org/10.1038/nrcardio.2010.162.

[88] Gurwitz JH, Field TS, Harrold LR, Rothschild J, Debellis K, Seger AC, et al. Incidence and Preventability of Adverse Drug Events Among Older Persons in the Ambulatory Setting. JAMA 2003;289:1107–16. https://doi.org/10.1001/jama.289.9.1107.

[89] Gwini SM, Coupland CAC, Siriwardena AN. The effect of influenza vaccination on risk of acute myocardial infarction: Self-controlled case-series study. Vaccine 2011;29:1145–9. https://doi.org/10.1016/j.vaccine.2010.12.017.

[90] Gurfinkel EP, de la Fuente RL. Two-Year Follow-Up of the FLU Vaccination Acute Coronary Syndromes (FLUVACS) Registry. Tex Heart Inst J 2004;31:28–32.

[91] Warren-Gash C, Smeeth L, Hayward AC. Influenza as a trigger for acute myocardial infarction or death from cardiovascular disease: a systematic review. Lancet Infect Dis 2009;9:601–10. https://doi.org/10.1016/S1473-3099(09)70233-6.

[92] Ciszewski A. Cardioprotective effect of influenza and pneumococcal vaccination in patients with cardiovascular diseases. Vaccine 2018;36:202–6. https://doi.org/10.1016/j.vaccine.2017.11.078.
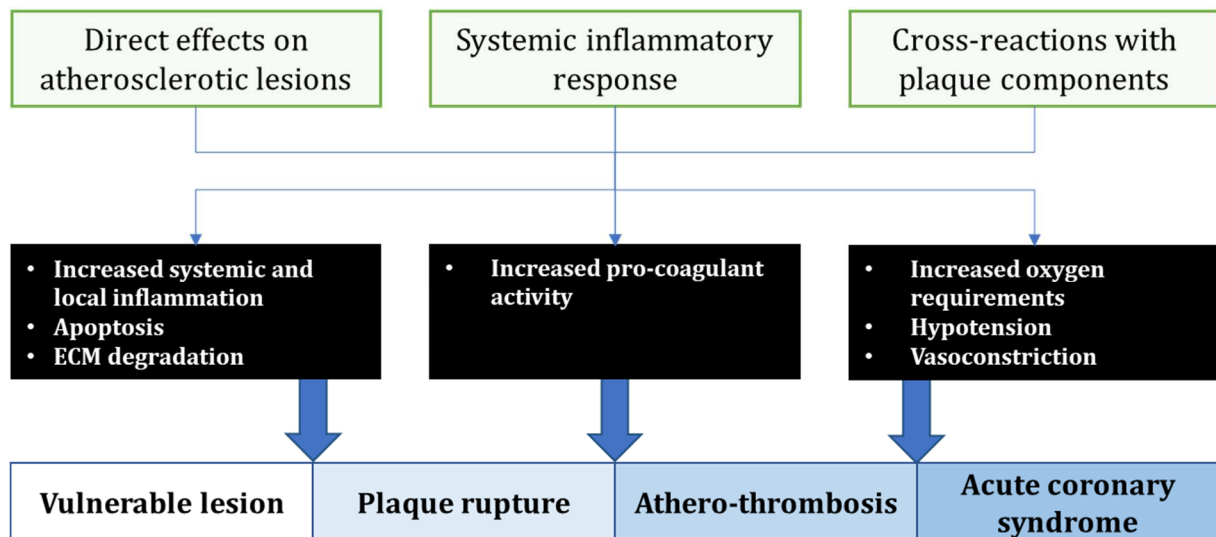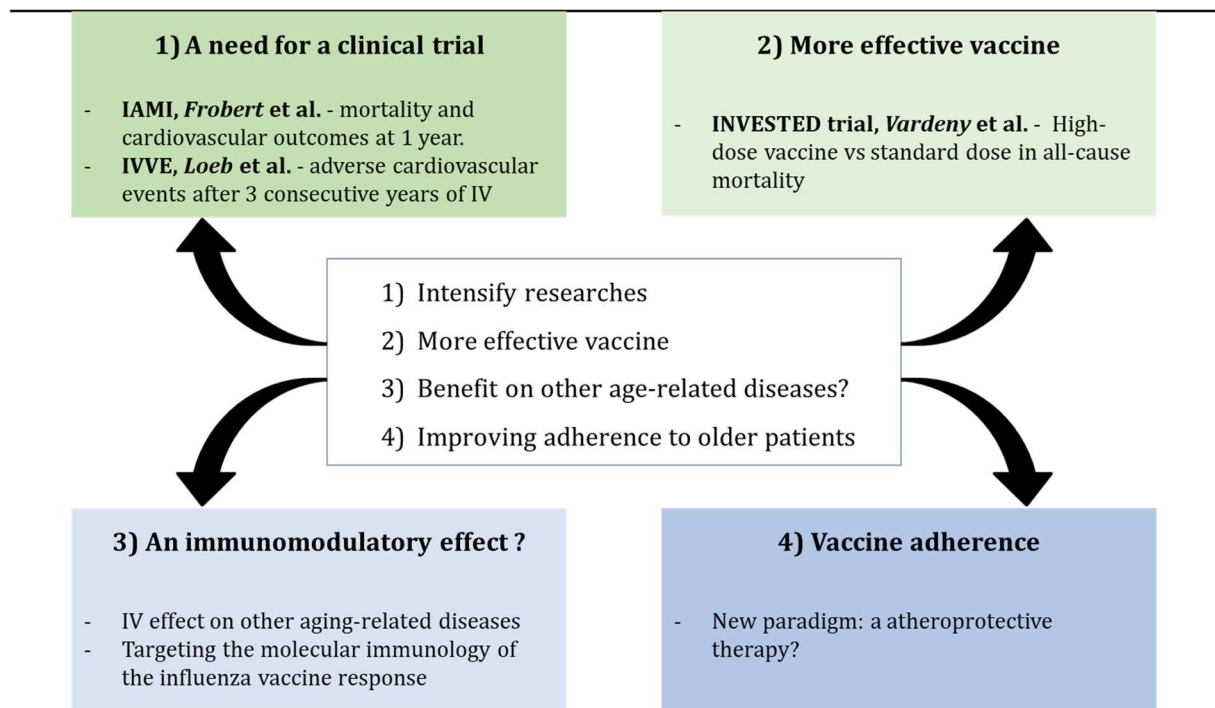
Figure 1 - Acute coronary syndrome triggered by influenza infection:

The influenza virus can induce, either by direct viral effect on atheromatous plaque or by indirect mechanisms (leading to a systemic inflammatory response), the destabilization of vulnerable plaque. However, both mechanisms do not appear to be specific to the influenza virus and similar data have been reported for CMV or Chlamydia pneumoniae infections. Antigenic similarities between the influenza virus and oxidized LDL have also been observed and could be responsible for cross-reactions, leading to disease progression.
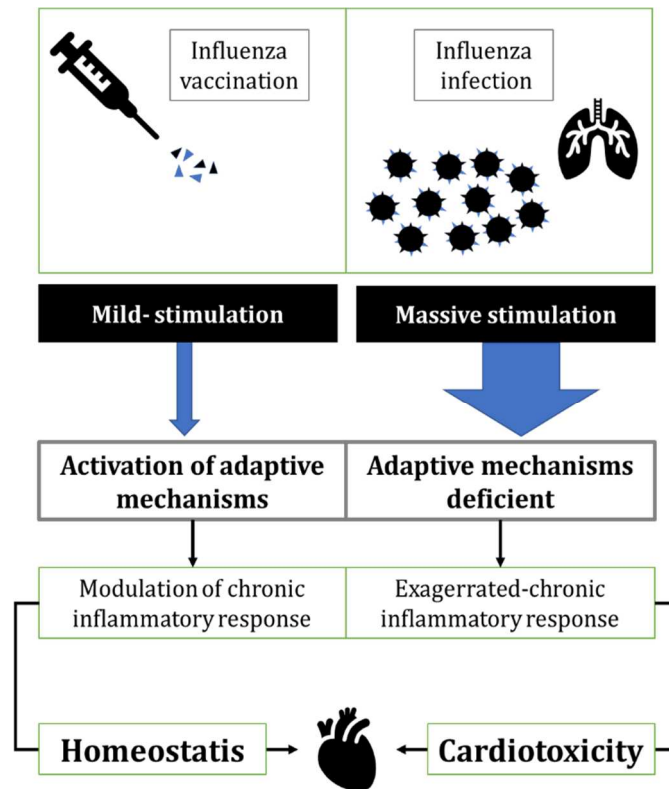
Figure 2: Schematic model of hormetic response to influenza vaccine and infection.

Hormesis, which can be summed up by the adage " that which does not kill us makes us stronger ", refers to a generally favourable stimulation response of biological defenses to low doses of stress-generating agents. Transposed to the issue of influenza vaccination and influenza infection, high stimulant stress, here influenza infection, induces damage to cells or organs, resulting in a deficient adaptive response leading to cardiotoxicity. A low stress of the same agent, the influenza vaccine, allows for an adaptive response that would confer protection against exposure to further severe stress.

1) A need for a clinical trial

- **IAMI, *Frobert* et al.** - mortality and cardiovascular outcomes at 1 year.
- **IVVE, *Loeb* et al.** - adverse cardiovascular events after 3 consecutive years of IV

2) More effective vaccine

- **INVESTED trial, *Vardeny* et al.** - High-dose vaccine vs standard dose in all-cause mortality

1) Intensify researches

2) More effective vaccine

3) Benefit on other age-related diseases?

4) Improving adherence to older patients

3) An immunomodulatory effect ?

- IV effect on other aging-related diseases
- Targeting the molecular immunology of the influenza vaccine response

4) Vaccine adherence

- New paradigm: a atheroprotective therapy?

Figure 3 – Future directions
Although studies on the association between influenza vaccination and cardiovascular risk prevention show limitations, they support efforts to increase research in this area. Influenza vaccination, because of its effectiveness in reducing cardiovascular events and complications of influenza, can be considered a new treatment in the arsenal of patients with coronary heart disease.
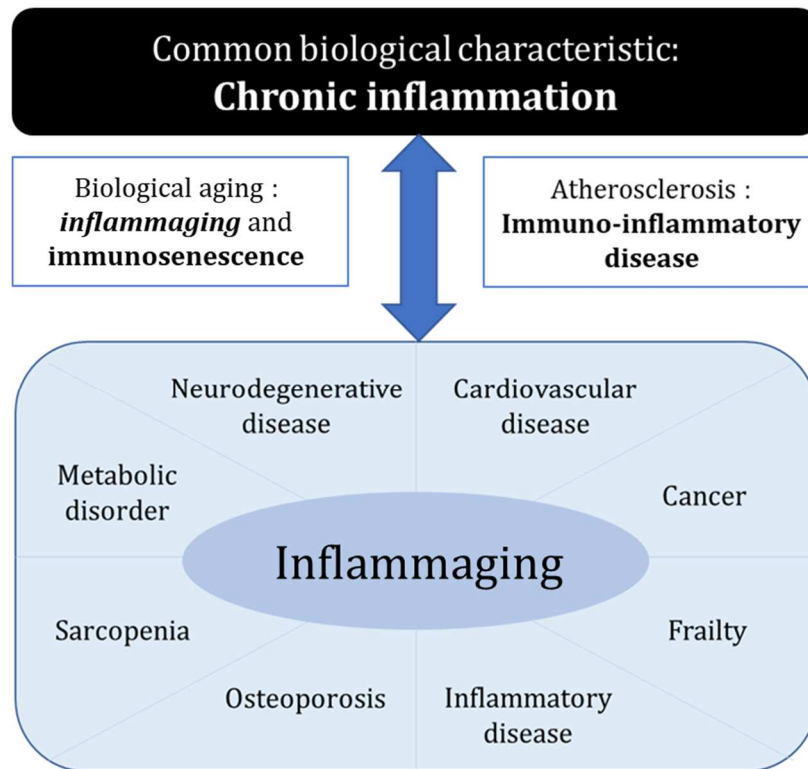
Figure 4 - Aging and aging-related diseases
Chronic inflammation is a common biological process that characterizes aging and aging-related diseases. It results in a systemic increase in pro-inflammatory biomarkers. However, its origin remains controversial, contributing both to the pathogenesis of aging-related diseases and reflecting their existence. It is therefore widely accepted that interdependence remains more consistent in explaining the interactions between inflammation and aging-related diseases.

Table 1: *Summary of relevant observational studies and clinical trials of the efficacy/effectiveness of influenza vaccination on the prevention of mortality and cardiovascular events in older adults.*

First author, year of publication	Study type	Follow-up	Strategy[a]	N	Age*† Vaccine	Age*† No vaccine	Study outcome	OR/HR†	Relevant additional outcomes (OR/HR)
Observational studies									
Liu., 2012 [38]	Cohort	4 y	II	5048	75±6	76±7	All-cause mortality **(1)**, hospitalization for CVD **(2)**	**(1)** 0.42** **(2)** 0.84 **	• Non-flu season: **(1)** 0.78**, **(2)** 1.04
Voordouw, 2004 [39]	Cohort	6 y	-	26071	73±7		All-cause mortality	0.76 **	• CV death: 0.89 • Non-flu season: 0.89 • RV effect with age: [65-69] 0.98, [70-79] 0.78**, [≥80] 0.69**
Armstrong, 2004 [42]	Cohort	4 y	-	24535	≥75		Daily all-cause mortality	0.89 **	• CV death, 0.87
Chen, 2016 [47]	Cohort	1 y	I	4406	71±8	68±10	Hospitalization for AMI	0.35 **	• RV effect: [1 IV] 0.62**, [2-3 IV] 0.35**, [≥4 IV] 0.13** • RV effect in non-flu season: [1] 0.61**, [2-3] 0.39**, [≥4] 0.13** • RV effect with age: [55-64] 0.47**, [65-74] 0.25**, [≥75] 0.42**
Hsu, 2016 [49]	Cohorts: **(1)** mismatched vaccine strain in 2007, **(2)** matched vaccine strain in 2008	1 y	-	202058	**(1)** *F* 75±6, *M* 75±6 **(2)** *F* 75±7, *M* 76±6	**(1)** *F* 75±7, *M* 75±7 **(2)** *F* 75±7, *M* 74±7	AMI	**(1)** *F* 1.10, *M* 0.99 **(2)** *F* 0.73, *M* 0.68**	
Vamos, 2016 [44]	Cohort	7 y	-	124503	66±13	56±16	Hospital admissions for: **(1)** AMI, **(2)** stroke, **(3)** HF	**(1)** 0.81 **(2)** 0.70 ** **(3)** 0.78 **	• Non-flu season: (1) 0.96, (2) 1.17, (3) 1.06
Nichol, 2003 [40]	2 flu seasons **(1)** 1998–1999, **(2)** 1999–2000	1 y	-	**(1)** 140055 **(2)** 146328	74±7 74±7	74±7 73±7	All-cause mortality	**(1)** 0.52 ** **(2)** 0.50 **	• AMI: (1) 0.80**, (2) 0.90 • Beneficial effect of IV with age: hospitalization for cardiac causes in 65-84 y.o. patients, all-cause mortality, hospitalization for stroke in 75-84 y.o. patients
							Hospitalization for cardiac disease	**(1)** 0.81** **(2)** 0.81 **	
							Hospitalization for stroke	**(1)** 0.84 ** **(2)** 0.77 **	
Wang, 2007 [41]	Population-based study	10 m		88731	≥65 y		Major cause-specific mortality	0.56 **	• Stroke: 0.35**, Heart disease: 0.78** • High-risk patients (with comorbidities): stroke: 0.25**, heart disease: 0.64**
Gwini, 2011 [89]	Self-controlled case-series	6 m	I	8180	77		AMI	<60 days: SS[b] > 60 days: NS[b]	• 1–14 days: 0.68** • Early IV effect (1st Sept-15th Nov): <60 days: SS[b] • No beneficial effect of late IV (16th Nov-30th April)
Heffelfinger, 2006 [45]	Case controlled	1 y	-	2485	72	73	AMI	0.97	• Non-flu season: 0.97
Naghavi, 2000 [37]	Case controlled	6 m	II	218	62±12	65±14	AMI	0.33 **	
Lavallée, 2002 [50]	Case controlled	5 y	-	270	72±7	73±7	Stroke	0.50 **	• RV for the last 5 years: 0.42** • No effect in ≥75 y.o. patients with 1 IV or RV for the last 5 years
Grau Armin, 2005 [46]	Case controlled	18 m	-	740	61±13	61±13	Stroke/transient ischemic attack	0.46 **	• Significant effects: men, older >65 yr, previous vascular diseases, ischemic stroke. • No effects in summer months.
Lin, 2014 [48]	Case controlled	5y	I	3120	75±7	75±7	Hospitalization for stroke	0.76 **	• Annual RV: 1 or 2y: 0.92, 3 or 4y:0.73, 5y:0.56** • No effects in summer months.
Chiang, 2017 [8]	Case controlled	13 y	I	160726	77±7	77±7	MACE (AMI, stroke)	0.80 **	• Non-flu season: 0.83** • RV effect: 0.78**

Study	Study type	Follow-up	Strategy[a]	N	Age *†		Study outcome	OR/HR†	Relevant additional outcomes (OR/HR)
					**Clinical trials and systematic reviews**				
Phrommintikul, 2011 [9]	RCT	12 m	II	439	65±9	67±9	MACE (death, hospitalization for ACS, HF, stroke)	0.70 **	• Hospitalization for ACS: 0.73**, HF: 0.69, CV death: 0.39
FLUCAD [10]	RCT	12 m	II	658	60±10		Cardiovascular death	1.06	• MACE (CV death, AMI, coronary revascularization): 0.54 • Coronary ischemic events (MACE, hospitalization for AMI): 0.54** • Beneficial effect in summer months
FLUVACS [11,12,90]	RCT	6 m	II	301	64	AMI 66, PCI 64	Cardiovascular death	0.25 **	• MACE (CV death, non-fatal MI, recurrent ischemia): 0.50**
		1 y		292				0.34 **	• MACE 0.59 **
		2 y		230	NR	NR		0.33	• Death, MI: 0.36
Warren-Gash, 2009 [91]	Meta-analysis	NR	-	NR	63		AMI	0.51	
Udell, 2013 [13]	Meta-analysis	7.9 m	-	6735	67		MACE (CV death, hospitalization for ACS, unstable angina, HF or stroke, and urgent coronary revascularization)	0.64 **	• More effective in patients with recent ACS: MACE, 0.45**, CV death 0.34** • CV death: 0.82 • No effect on individual nonfatal cardiovascular events • No beneficial effect with experimental IV

Abbreviations: AMI, acute myocardial infarction, CV, cardiovascular, CVD, cardiovascular disease, F, female, HF, heart failure, IV, influenza vaccination, M, male, MACE, major adverse cardiovascular events, NR, not reported, NS, not significant, PCI, planned angioplasty/stent, RCT, randomized controlled trial, RV, repeat vaccination

* Mean ± SD (years), unless otherwise specified.

** the 95%CI did not include the null value

† Rounded to the nearest integer

§ Efficacy reported for a statistically significant OR/HR

[a] Specific preventive strategy (primary prevention (I) or secondary prevention (II)). Unless otherwise specified, prior CVD was not an exclusion criterion.

[b] Summary of primary outcomes by follow-up period, for more relevance

Table 2 – *Summary and characteristics of recent studies of mechanisms possibly involved in a specific atheroprotective effect of IV.*

Study	Design	Materials or study population	Influenza strain or vaccine	Study objective
Veljkovic et al. (2014) [54]	Molecular study *Informational spectrum method for analysis of protein–protein interactions*	Databases from GenBank and GISAID (influenza virus), UniProt database (human proteins)	Hemagglutinin influenza A vaccine	To identify hemagglutinin antibody cross-reactivity in the atheroprotective signaling pathway
Bermudez-Fajardo et al. (2011) [51]	Animal study *Inoculation of increasing doses of influenza, pneumococcal and placebo vaccines*	Male ApoE$^{-/-}$ mice, 8 weeks old.	Vaxigrip®	To evaluate the effect of influenza vaccination on atherosclerotic lesion shape and its inflammatory component.
Keshtkar-Jahromi et al. (2018) [52]	Prospective observational study of community-dwelling adults, 2007–2008.	▪ 70 y and over ▪ Mean ± SD age: 84.6 ± 4.6	Fluarix®	To determine the association of vaccine-induced strain-specific antibody responses and frailty with post-vaccination levels of tumor necrosis factor-alpha-like weak inducer of apoptosis
Ciszewski et al. (2018) [92]	Literature review	Not applicable	Not reported	To summarize the potential cardioprotective effect of IV on atherosclerosis