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1 **Influenza vaccination as a novel means of preventing**
2 **coronary heart disease: effectiveness in older adults**

3
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20

21 **Abstract**

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

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

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

41 Aging is characterized by chronic inflammation (sometimes referred to as “inflammaging”) that
42 progresses insidiously during the course of aging-related diseases, including CVD. It remains
43 to be determined whether vaccination has an effect on aging-related diseases other than CVD.

44 Although the studies of this topic had various limitations, the results highlight the potential
45 benefits of vaccination in protecting the health of older adults, and should drive research on the

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

48

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

50

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

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70 **Introduction**

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

78 After CVD, respiratory tract infection (including influenza) is the third-ranked cause of death
79 worldwide. It has been estimated that each year, 290,000 to 650,000 deaths worldwide are
80 influenza-related [2]. A growing body of epidemiological and biological data suggests that
81 influenza infection is a risk factor for coronary heart disease (CHD). The elevated
82 cardiovascular mortality in winter and its association with the influenza disease peak [3], the
83 local and systemic inflammatory mechanisms induced by the virus [4–7], and the virus’s
84 selective presence in atherosclerotic lesions in animal and human vessels [4,5] have prompted
85 researchers to consider the influenza virus as a risk factor for atherosclerosis progression. The
86 possibility that the influenza virus triggers CHD complications has prompted a resurgence of
87 interest in influenza vaccination (IV). Secondary prevention studies of older adults have shown
88 that IV is associated with a reduction in the incidence of major adverse cardiovascular events
89 [8–13]. Thus, IV might be of particular value in older adults at a high risk of influenza infection
90 and CHD complications. Understanding the underlying cellular processes is likely to be crucial
91 in the development of new strategies for preventing and/or treating cardiovascular
92 complications.

93

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

98

99 **1 Cardiovascular disease and aging**

100

101 *1.1 Atherosclerosis*

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

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

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

120

121 *1.2 Arterial aging*

122 Arterial aging is a key factor in the incidence and prevalence of CVD. It leads to structural,
123 functional, and mechanical arterial remodeling, even when other CVD risk factors are absent.

124 Although arterial aging is a normal physiological process, aged arteries are characterized by
125 endothelial dysfunction, vascular remodeling, inflammation, calcification, and increased
126 stiffness – all of which are also characteristics of atherosclerosis. Aged vessels express elevated
127 levels of pro-inflammatory mediators and take up plasma LDL more readily. These effects may
128 result in greater expression of leukocyte adhesion molecules on endothelial cells in aged
129 vessels, which would induce monocyte migration, greater uptake of atherogenic lipoproteins,
130 and subsequent inflammation – all key events that ultimately promote atherosclerosis [17,18].

131 The direct relationship between aging and vascular health is also evident in progeria, a
132 syndrome in which patients present accelerated aging – including accelerated atherosclerosis
133 and premature cardiovascular mortality [19]. The boundaries between arterial aging and
134 atherosclerosis are difficult to establish; hence, these two entities should be considered on a
135 continuum, with synergistic actions on the cardiovascular risk.

136

137 **2 Cardiovascular disease, inflammation, and the influenza virus.**

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

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

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

149

150 2.1 The influenza virus and atherosclerosis

151 An association between influenza infection and acute coronary syndrome (ACS) was first
152 suggested during the influenza epidemic that struck Europe and the United States in the early
153 1900s. During these epidemics, more than half of all deaths were attributed to a cause other
154 than influenza, and CVD was predominant [22]. The excess cardiovascular mortality in winter
155 and its association with the influenza peak strongly suggested a relationship between influenza
156 infection and ACS [3]. In a large cohort of older adults, the risk of myocardial infarction and
157 stroke were found to be significantly higher during the first three days following the diagnosis
158 of a respiratory infections (incidence ratio for ACS, 4.95; 95%CI [4.43-5.53] and for stroke,
159 3.19; 95%CI [2.81 to 3.62]) [23]. More recently, influenza infection has been linked to a higher
160 risk of cardiovascular mortality within 14 days, and was strongly correlated with fatal ACS
161 (with an increase in incidence ranging from 5.8% (95%CI [2.5-9.1%]) to 13.1% (95%CI [5.3-
162 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].

212 The viral hemagglutinin's binding zone and the apolipoprotein B in LDL particles display
213 significant molecular similarities [34]. The influenza virus might therefore affect lipid
214 metabolism by modulating ongoing immune and inflammatory processes during
215 atherosclerosis. A positive correlation between the respective titers of anti-influenza A
216 immunoglobulin (Ig)G antibodies and anti-oxidized LDL antibodies has been demonstrated in
217 patients with documented progression of atherosclerosis [30]. In the mouse, influenza A virus
218 infection was associated with a loss of high-density lipoprotein anti-inflammatory properties
219 [35], promoting macrophage traffic into arteries [36]. Consequently, the loss of high-density
220 lipoprotein's protective effect might increase LDL oxidation, atherosclerosis progression, and
221 plaque destabilization. Gurevich et al. have hypothesized several possible autoimmune
222 mechanisms: (i) the systemic inflammatory response to infection may enhance lipid
223 peroxidation and the subsequent production of autoantibodies against modified LDL, (ii) direct
224 vessel wall colonization may initiate a local cell-based autoimmune reaction via activation of
225 antigen-presenting cells; and (iii) molecular mimicry may stimulate an atherogenic autoimmune
226 reaction that prompts the development and progression of atherosclerosis.

227

228 **3 Beneficial effects of influenza vaccination**

229

230 *3.1 Influenza vaccination prevents cardiovascular events in older adults*

231 Although it is still difficult to establish a causal relationship between influenza infection and
232 ACS on the basis of epidemiological data, the available evidence has prompted physicians to
233 try a therapeutic approach using IV. Naghavi et al. were among the first to hypothesize that IV
234 might protect against incident ACS. In their study population (mean age: 62 years), IV in the
235 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

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

269 **The influenza-infection-independent mechanism.**

270 Few studies of an influenza-infection-independent mechanism have been performed (**Table 2**).
271 Bermudez-Fajardo *et al.* were the first to study infection-independent effects on atherosclerotic
272 lesions in the ApoE^{-/-} mouse. At high doses, IV modulated the T-cell inflammatory response
273 in fibrolipidic lesions, and was associated with stable plaques, a decrease in the secretion of
274 pro-inflammatory cytokines (interferon gamma, IL-2, and IL-17), and an increase in the
275 secretion of anti-inflammatory cytokines (IL-4) [51]. However, the underlying mechanism
276 could not be determined. A time and dose-dependent increase in anti-influenza IgG1 levels in
277 immunized mice suggesting that IV's protective effect with regard to CVD might be antibody-
278 dependent. The impact on the cytokine response has been assessed by Keshtkar-Jahromi *et al.*
279 [52]. The pro-inflammatory cytokine tumor necrosis factor-alpha-related weak inducer of
280 apoptosis (TWEAK) is produced by peripheral blood monocytes and contributes to the
281 progression of atherosclerosis. Its activities include a proatherogenic response, the proliferation
282 and migration of smooth muscle cells in atherosclerotic plaques, and increased synthesis of
283 metalloproteinases (thus reducing plaque stability) [53]. In a study of 69 community-dwelling

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; $\chi^2=10.6$, 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

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

340

341 **4 Discussion**

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

348

349 **Aging and influenza vaccination**

350 Aging is accompanied by immunosenescence, increased susceptibility to infection, and reduced
351 responses to vaccination. Studies of geriatric populations have largely identified the age over
352 65 as a criterion for defining older persons. In the field of geriatric medicine, it is widely
353 accepted that an aging phenotype is a better criterion for physiologic age than chronological
354 age *per se*. The prevalence of frailty (a geriatric syndrome marked by loss of function and
355 physiological reserve) rises with age. It has been suggested that frailty is a causative and
356 prognostic factor for CVD. In a meta-analysis of 54,250 older adults, CVD was associated with

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

366 **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.

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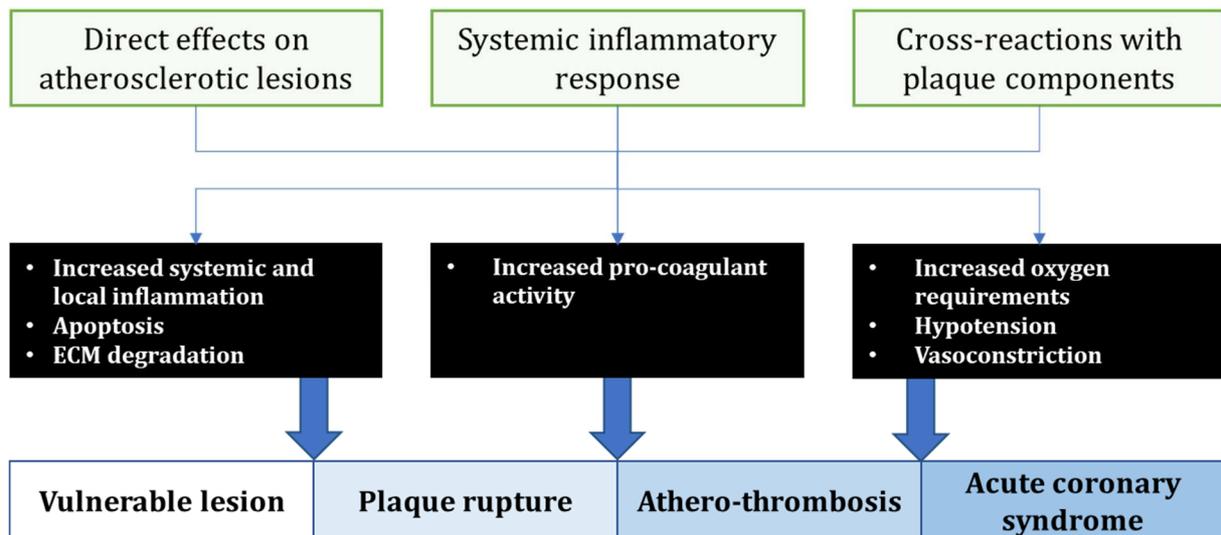


Figure 1 - Acute coronary syndrome triggered by influenza infection:

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.

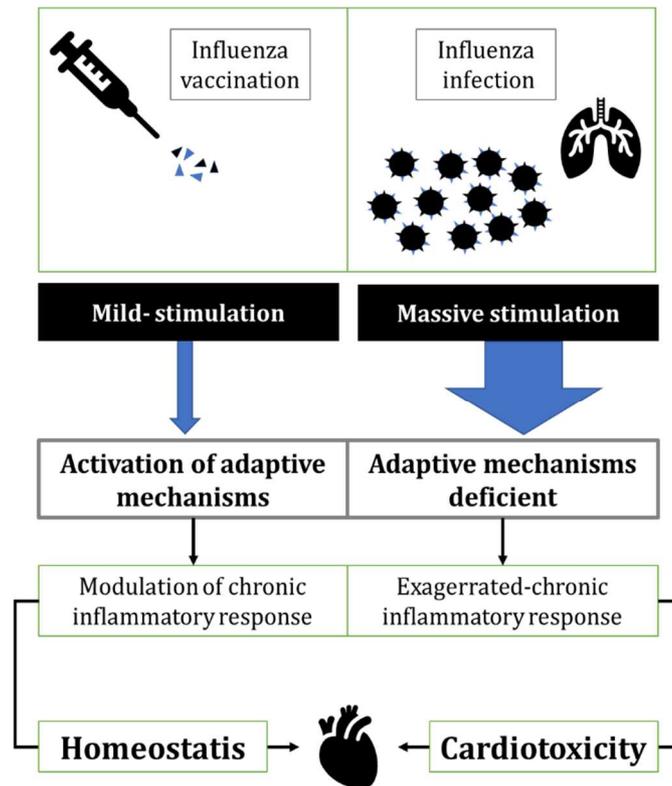


Figure 2: Schematic model of hormetic response to influenza vaccine and infection.

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.

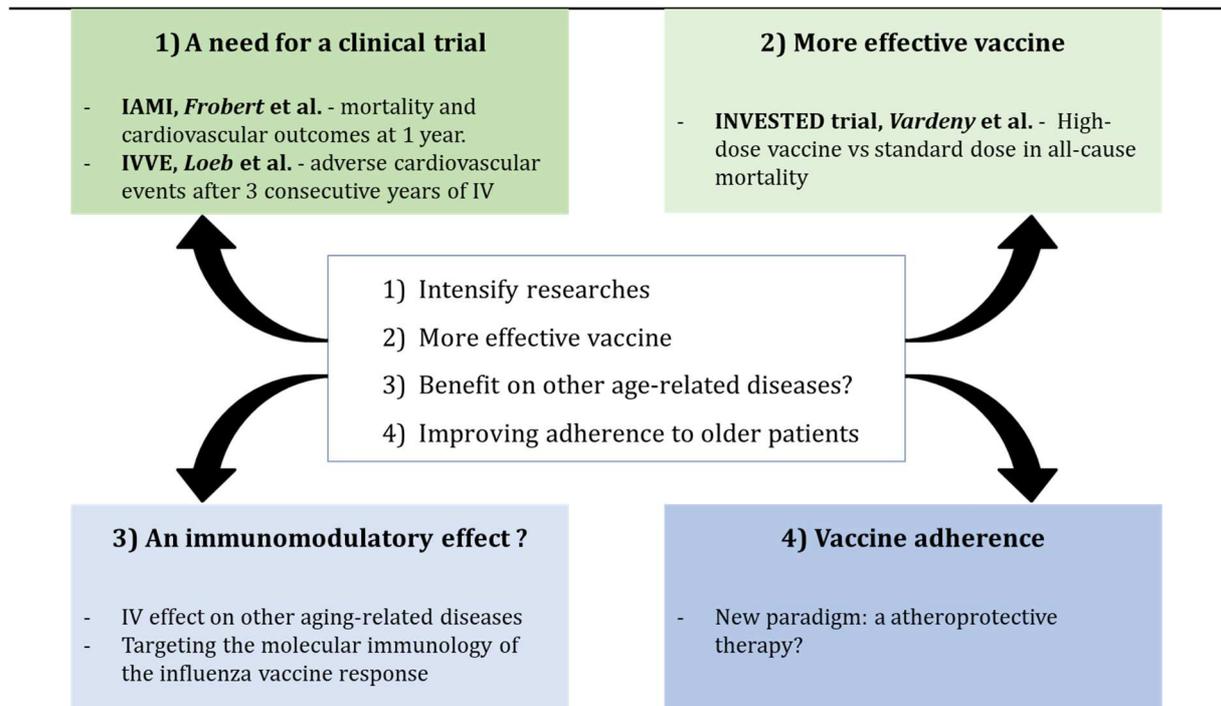


Figure 3 – Future directions

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.

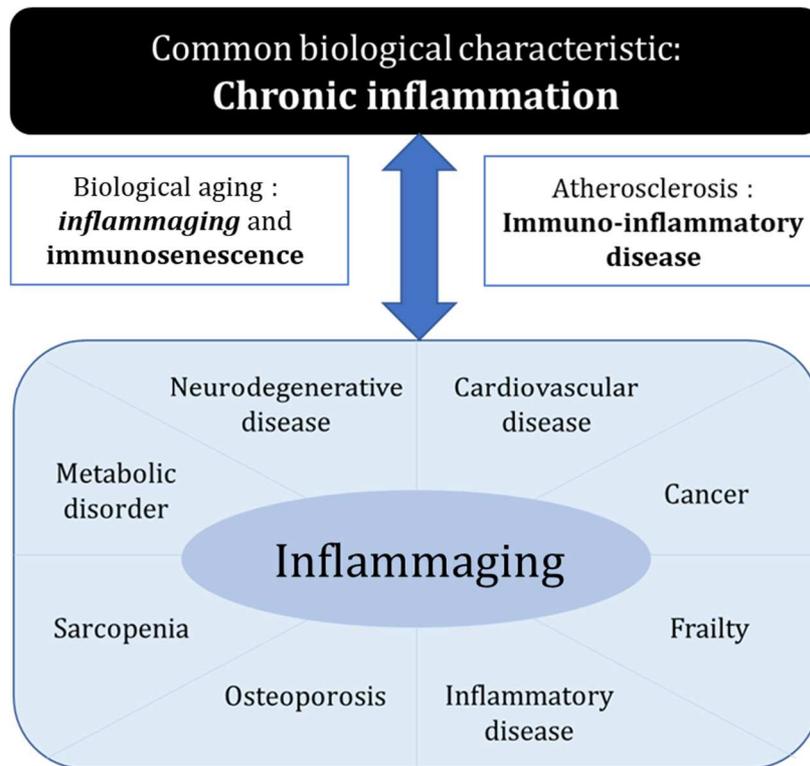


Figure 4 - Aging and aging-related diseases

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*†		Study outcome	OR/HR†	Relevant additional outcomes (OR/HR)
					Vaccine	No vaccine			
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 **	<ul style="list-style-type: none"> Non-flu season: (1) 0.78**, (2) 1.04
Voordouw, 2004 [39]	Cohort	6 y	-	26071	73±7		All-cause mortality	0.76 **	<ul style="list-style-type: none"> 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 **	<ul style="list-style-type: none"> CV death, 0.87
Chen, 2016 [47]	Cohort	1 y	I	4406	71±8	68±10	Hospitalization for AMI	0.35 **	<ul style="list-style-type: none"> 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 **	<ul style="list-style-type: none"> 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 **	<ul style="list-style-type: none"> 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 **	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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 **	<ul style="list-style-type: none"> 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 **	<ul style="list-style-type: none"> 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 **	<ul style="list-style-type: none"> 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 **	<ul style="list-style-type: none"> 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 **	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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 **	<ul style="list-style-type: none"> MACE (CV death, non-fatal MI, recurrent ischemia): 0.50** MACE 0.59 ** Death, MI: 0.36
		1 y		292				0.34 **	
		2 y		230				NR	
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 **	<ul style="list-style-type: none"> 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 <i>Informational spectrum method for analysis of protein–protein interactions</i>	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 <i>Inoculation of increasing doses of influenza, pneumococcal and placebo vaccines</i>	Male ApoE ^{-/-} mice, 8 weeks old.	Vaxigrip®	To evaluate the effect of influenza vaccination on atherosclerotic lesion shape and its inflammatory component.
Keshkar-Jahromi et al. (2018) [52]	Prospective observational study of community-dwelling adults, 2007–2008.	<ul style="list-style-type: none"> ▪ 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