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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

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].

Studies of patients with laboratory-confirmed influenza infections have underlined the virus's involvement in the atherosclerotic process. In one study, serologically confirmed influenza infection was significantly more frequent in patients with recent ACS [25]; however, this was not a predictive factor in MacIntyre et al.'s case control study [26]. A recent self-controlled case-series (median patient age: 77 years) revealed that laboratory-confirmed infection was associated with a 6-fold higher risk of hospital admission for ACS [27]. Although many pathogens have been studied as putative risk factors in CHD, the epidemiological data suggest that the influenza virus is the most serious candidate for involvement in the atherosclerotic process.

2.2 *Acute coronary syndrome triggered by the influenza virus*

The results of animal studies have provided a pathophysiological explanation for the influenza virus's putative involvement in the atherosclerotic process. Influenza infection might trigger ACS in one or more of three ways: (i) *a direct viral effect* of the virus present in the atherosclerotic plaque, (ii) *an indirect inflammatory effect* due to a systemic inflammatory response to infection, and (iii) *a direct cross-reactive effect,* related to cross-reactions between viral antigens and proatherogenic plaque components (**Figure 1**).

The presence of a **direct effect** is suggested by the virus's vascular tropism and its association with lesion progression. In the $ApoE^{-/-}$ mouse model of atherosclerotic disease, influenza A virus was found to infect and specifically reside in fibro-lipid plaques – even in the absence of apparent viremia [4]. Furthermore, the presence of the virus was associated with an exacerbated local inflammatory response involving macrophage infiltration and a systemic pro-inflammatory cytokine response. Furthermore, $ApoE^{-/-}$ mice inoculated with a lethal dose of 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

influenza season (OR 0.33; 95%CI [0.13-0.82]; *p*=0.017) [37]. Thereafter, many observational studies evidenced a beneficial effect of IV on mortality and on acute ischemic events (**Table 1**). Although the effectiveness of IV in reducing all-cause mortality in older adults appears to be well established [38–42], its effect on specific cardiovascular mortality [9–13,39,41,42] and the degree of protection are subject to debate. Gross et *al*. estimated that the risk from 27–30% lower in case-control studies and 56–76% lower in cohort studies [43]. In contrast, Udell et al.'s meta-analysis (mean age of the analyzed patients: 67) failed to find a significant effect on cardiovascular mortality [13]. Although the epidemiological data suggest that a beneficial effect may not be present during the summer months [8,10,38,39,44–48] and may not be provided by matched/mismatched vaccine strains in older adults [49], the results of the FLUVACS and FLUCAD early randomized trials consistently evidenced an association between mortality and ischemic events outside the influenza season [10–12].

A number of studies have found that IV also protects against major non-fatal cardiovascular events, with a significantly lower risk of hospitalization for heart disease [9,13,38,40], AMI [8,9,13,40,47] and stroke [8,40,50] in older adults. Observational and clinical studies still show discordant results with regard to the protective association with AMI, although the association with stroke is more consistent **(Table 1)**. Lastly, repeat vaccination shows a consistent, dose-dependent, protective association with ACS [8,47] and stroke [8,48,50]. Although there are still some gray areas, the studies of repeat vaccination fit with the observational studies and emphasize the need for more robust clinical research on the beneficial effects of IV in older adults.

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

older adults, serum levels of TWEAK were found to be significantly and abnormally low four weeks after Fluarix® IV, and the effect was greatest in frail individuals. Furthermore, this decrease was inversely proportional to the vaccine-induced antibody response and was not associated with monocyte/macrophage activation – suggesting that the beneficial effect of IV on circulating TWEAK levels had been induced by the vaccine directly or had been mediated by a monocyte-independent pathway.

Using the informational spectrum method, Veljko et al. suggested that vaccine antibodies could act as agonists on atheroprotective pathways. A comparative structural analysis indicated the involvement of the bradykinin B2 receptor (BKB2R), which has a key role in cardiovascular homeostasis [54]. Bradykinin's interaction with its receptor induces an antioxidant and anti-inflammatory response [55], including beneficial coronary dilatation in early-stage ACS and during remodeling after AMI. Furthermore, activation of the BKB2R pathway inhibits apoptosis, inflammation, and myocardial hypertrophy [55]. Lastly, identification of antigenic similarities between viral antigens and oxidized LDL suggested that a cross-reaction was possible [34]. One can hypothesize that the antibodies elicited by IV bind to oxidized LDL and prevent it from being internalized by macrophages – thus limiting the development and progression of atherosclerosis. However, this mechanism is has yet to be demonstrated in practice, and further research is warranted.

3.3 Influenza vaccination and infection: paradoxical responses

It has been suggested that IV can serve as an *in vivo* model of the mild inflammatory stimulation [76]. Influenza vaccination causes transient changes in several biomarkers of inflammation and lipid status. In healthy older adults, the serum CRP level increases slightly but significantly 1 to 3 days after IV [56]. In patients with carotid stenosis, the CRP level after IV was 1.3 times

higher than in healthy controls (95% CI, 0.84–2.02, P=0.240) [57]. Increased serum acute-phase protein levels have been linked to atherosclerotic plaque instability and ACS. In a meta-analysis of more than 7000 patients with coronary events, subjects with a serum CRP level in the upper tertile had a higher risk of acute cardiovascular events (combined OR 1.49, 95% CI, 1.37 to 1.62; χ^2=10.6, P=0.01) [58].

If exaggerated inflammation after an influenza infection exacerbates atherosclerotic lesions, why does IV have the opposite effect? And why does influenza infection not confer the same long-term protection as IV?

We suggest that these apparently paradoxical observations can be explained by the concept of hormesis, whereby a low dose of a stressor initiates compensatory biological processes, activates adaptive systems, and confers protection against subsequent exposure to a high dose of the same stressor (**Figure 2**). This phenomenon is also referred to as ischemic preconditioning and is widely recognized in the field of CVD. In an animal study, the myocardial infarction size was 75% smaller after four prior 5-minute periods of ischemia separated by 5 minutes reperfusion periods [59]. Thus, a "sub-damaging" stimulus confers resistance to ischemic heart injury. Influenza vaccination induces a mild, transient systemic inflammatory response, which may well explain why this vaccination is not associated with an increased risk of ACS. The results of clinical and animal studies suggest that hormesis can protect against many aging-related diseases, including diabetes, CVD, cancers and neurocognitive disorders [60].

Furthermore, a wide variety of stimuli (such as thermal stress [61], bradykinin, and nitric oxide (NO) [60]) can trigger the protective signal. As mentioned above, Veljko et *al.* highlighted similarities between an anti-hemagglutinin A1 antibody and bradykinin B2, with the potential for agonism on the BKB2R [55]. At the endothelial level, activated BKB2R induces NO release 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

an OR of between 2.7 and 4.1 for prevalent frailty and of 1.5 for incident frailty in those who were not frail at baseline [65]. Regardless of age, the severity of the underlying disease, comorbidities, and disability, frailty is a powerful predictor of mortality in patients with CVD. In older adults with severe CHD or heart failure, the prevalence of frailty ranged from 50% to 54%, and was associated with an increased risk of all-cause mortality (OR from 1.6 to 4.0) [65].

As frailty is an independent risk factor for CVD in older adults, it will be important to consider this variable in future studies of vaccine efficacy in older subjects and is of crucial importance in understanding the cardiovascular health benefits of IV.

Vitamin D: a potential adjuvant?

Vitamin D is an important factor in cardiovascular health. In epidemiological studies, vitamin D deficiency is consistently associated with an higher risk of CVD [66]. *In vitro* studies have shown that vitamin D suppresses inflammation, reduces the internalization of ox-LDL, decreases the expression of many pro-inflammatory cytokines, and polarizes T cells towards a Th2 response [74,76]. However, RCT and meta-analysis findings regarding the beneficial effects of vitamin D supplementation (standard dose, high dose and/or their association with calcium supplementation) on CVD outcomes have remained largely disappointing. [67,68]. Two of the factors limiting assessment of the effect of vitamin D are baseline status and efficacy of supplementation. The definition of hypovitaminosis D and the "optimal" status of vitamin D is still controversial in vitamin D research [69]. In addition, the administration of 25 (OH) D is often only based on tests that still need to be standardized to allow for the pooling of research data [70].

Vitamin D deficiency affects nearly 50% of older adults in Europe, and its prevalence increases with age [71]. The association between low vitamin D levels and frailty has been evaluated in several studies. A recent systematic review with meta-analysis found that people with low serum levels of 25 (OH) D had a higher risk of frailty [72]. This association may be explained by the effect of vitamin D on bone health and muscle strength.

Vitamin D evaluated in the context of the IV did not find a correlation between 25-(OH) D levels and response to the vaccine. In a recent clinical trial, vitamin D supplementation in older adults who were deficient at baseline (<30 ng/mL) and then vaccinated was not associated with a difference in the antibody response but interestingly, was associated with lymphocyte polarization towards a Th2 tolerogenic immune response, supporting the immunomodulatory effect of vitamin D [73].

The link between vitamin D deficiency, cardiovascular disease, and frailty reinforced the need to define geriatric populations in a different way than age and to consider vitamin D status in the assessment of CVD in future studies targeting older people. Moreover, future studies should help to determine whether vitamin D supplementation associated with IV prevents CVD outcomes in older adults with different basal serum 25-(OH) D levels.

5 Research perspectives

The results reviewed above emphasize the need for further research on IV and notably its potential value in preventive medicine and its benefits in older adults **(Figure 3).**

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]**.

This pathogenesis common to age-related diseases leads us to consider a pleiotropic effect of the vaccine as additional evidence of an immunomodulatory effect. To the best of our knowledge, only a few studies have examined the effect of IV on other aging-related diseases. Prior exposure to diphtheria, tetanus and polio vaccines (but not influenza vaccine) was associated with a lower risk of Alzheimer's disease in older adults (OR [95%CI] = 0.41, [0.27–0.62], 0.60 [0.37–0.99], and 0.75 [0.54–1.04], respectively) [80]. Furthermore, IV was associated with significantly lower major-cause mortality for stroke (hazard ratio (HR)=0.35), renal disease (HR=0.40), diabetes mellitus (HR=0.45), pneumonia (HR=0.47), and cancer (HR=0.74) [41]. Selectively stimulating the atheroprotective immune response while inhibiting pathogenic immune responses following influenza vaccination offers new and interesting therapeutic possibilities.

Second, few studies have focused on the specific mechanistic processes that underlie IV (**Table 2**). The heterogeneity of these studies' approaches and designs weakens their coherence and validity, and prevents firm conclusions from being drawn. Possible antigenic mimicry between influenza A hemagglutinin (HA) and apolipoprotein B-100 (ApoB-100) [30,34] is a promising putative mechanism. Both native and aldehyde-modified ApoB-100 are important targets for protective responses in atherosclerosis. Anti-apoB-100 antibodies induced a 40 to 70% decrease in atherosclerosis and inflammation in animal models [81]. A transient anti-inflammatory Th2 response (mediated by anti-ApoB-100 IgG1) was associated with a relative decrease in the size of atherosclerotic lesions in the ApoE $^{-/-}$ mouse, as is also observed after IV. We suggest that the ApoB-100 peptide might be a valuable tool for understanding cellular processes after vaccination.

Scientific interest in IV and the possibly associated cardiovascular protection continues to grow. Although clinical data are gradually strengthening this association, there is a marked lack of molecular immunology studies in this field. The generation of additional biological data will

justify greater efforts for defining IV as an atheroprotective measure and for expanding current treatment options. Confirmation of IV's cardioprotective effect and characterization of the underlying mechanism might lead to greater vaccination coverage and synergistic preventive effects on influenza infection and CVD.

5.3 Development of a more effective influenza vaccine

There is a great need for vaccines that optimally stimulate the immune system of older adults. A meta-analysis of more than 30 vaccine studies showed that the clinical effectiveness ranged from 70 to 90% in young adults, and from 17 to 53% in older adults [82]. To increase effectiveness, several optimized vaccination strategies have been developed; these include intradermal vaccines, high-dose vaccines, and the adjuvant oil-in-water emulsion vaccine MF59. In older adults, all these formulations are slightly more immunogenic than standard trivalent inactivated vaccines. In a meta-analysis of studies in older adults, a high-dose vaccine was significantly more effective (relative vaccine efficacy, 17.7%; 95% CI, 6.6–27.4%) in reducing major cardiorespiratory events, relative to a standard dose vaccine [83]. However, the results were discordant in subgroup analyses of hospitalizations due to ACS and strokes. Similarly, the MF59 adjuvanted vaccine was associated with a significant reduction in hospitalizations for ACS and stroke (adjusted OR 0.13; 95% CI 0.03–0.65 and adjusted OR 0.07; 95% CI 0.01–0.48 respectively) during viral circulation, relative to non-vaccinated patients [84]. The establishment of new vaccination protocols is needed to prevent the incidence of infectious diseases and reduce the associated mortality. In this perspective, Vardeny et *al.* are conducting a randomized controlled trial (the INVESTED trial) of a high-dose vaccine and the standard vaccine; a lower incidence of cardiovascular adverse events with the high-dose

vaccine would suggest a dose-response relationship between IV and cardiovascular prevention [85].

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

Interventions aimed at preventing CVD (including lifestyle interventions, risk factor control, and medications such as beta-blockers, aspirin/clopidogrel, angiotensin II receptor blockers, and statins) are as effective in older adults as they are in younger adults. However, these strategies are under-used in older adults [86,87]. Effective intervention is complicated by ageism, comorbidities, and limited access to age-appropriate care. Adverse drug reactions (although often preventable) are frequent. Cardiovascular medications were the medications most frequently implicated (26.0% of cases) in adverse reactions in older adults [88].

Recognition of the beneficial effect of IV offers a new therapeutic opportunity for preventing CVD. Vaccination is a well-tolerated, inexpensive, effective method for reducing morbidity in patients at high risk of CVD. Vaccination has a favorable cost/benefit ratio, when compared with current pharmacological treatments [26]. A paradigm shift may be necessary to encourage clinicians to see IV as a cost-effective, safe, adjunct prevention strategy for patients with CVD.

Conclusion

Cardiovascular disease and influenza infection are leading causes of death worldwide, and the disease burdens are greater in people over the age 65. There is growing evidence of a link between IV and a reduction in the incidence of cardiovascular events. This is firstly based on prevention of the cardiovascular complications of influenza infection. We also suggest that IV

has other infection-independent cardioprotective effects. The scientific interest in IV and its cardiovascular protection is and continues to grow. However, its specific effect remains largely controversial and difficult to share despite promising results. The provision of additional biological data will justify a greater investment in order to define IV as an atheroprotective vaccine.

Authors' contributions

AA and BF made substantial contributions to study conception and design. AA wrote the manuscript. AA, JM, DA, CD, GG and BF have made substantial contributions to the final manuscript. All authors read and approved the final manuscript.

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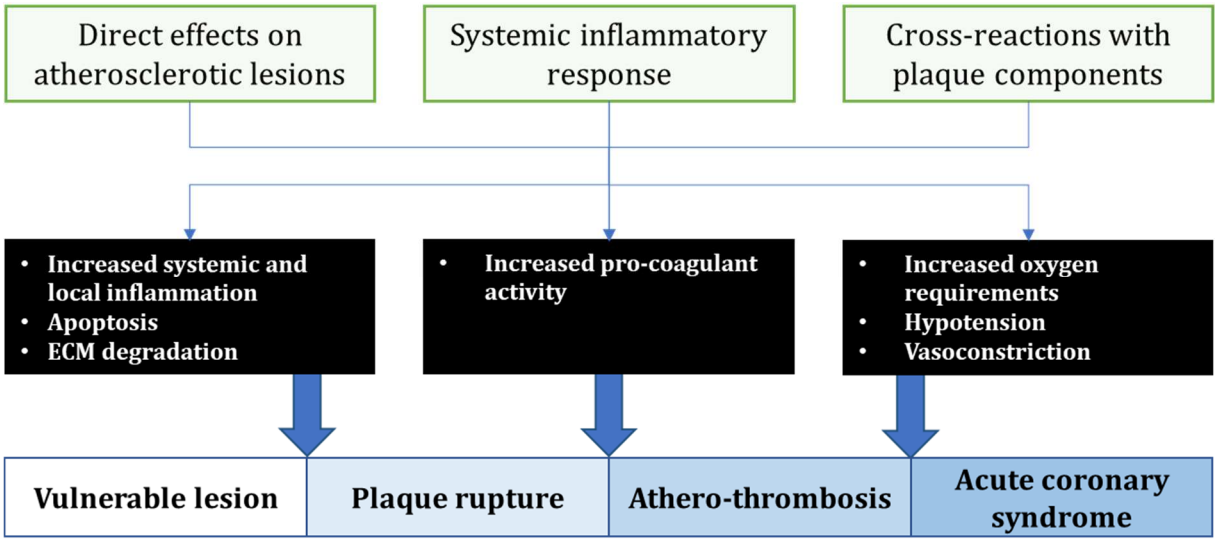

Figure 1 - Acute coronary syndrome triggered by influenza infection:

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.

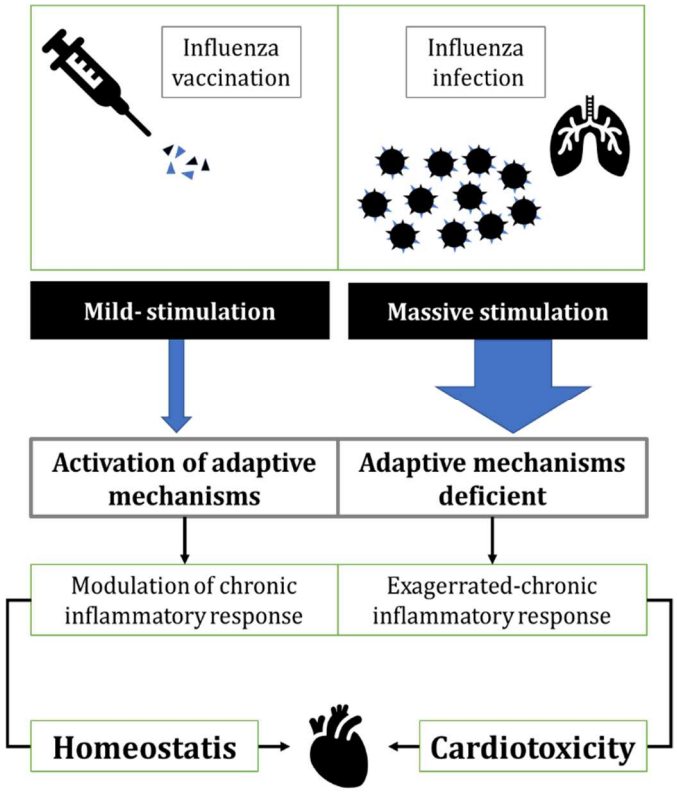

Figure 2: Schematic model of hormetic response to influenza vaccine and infection.
Hormesis, which can be summed up by the adage " that which does not kill us makes us stronger ", refers to a generally favourable stimulation response of biological defenses to low doses of stress-generating agents. Transposed 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.

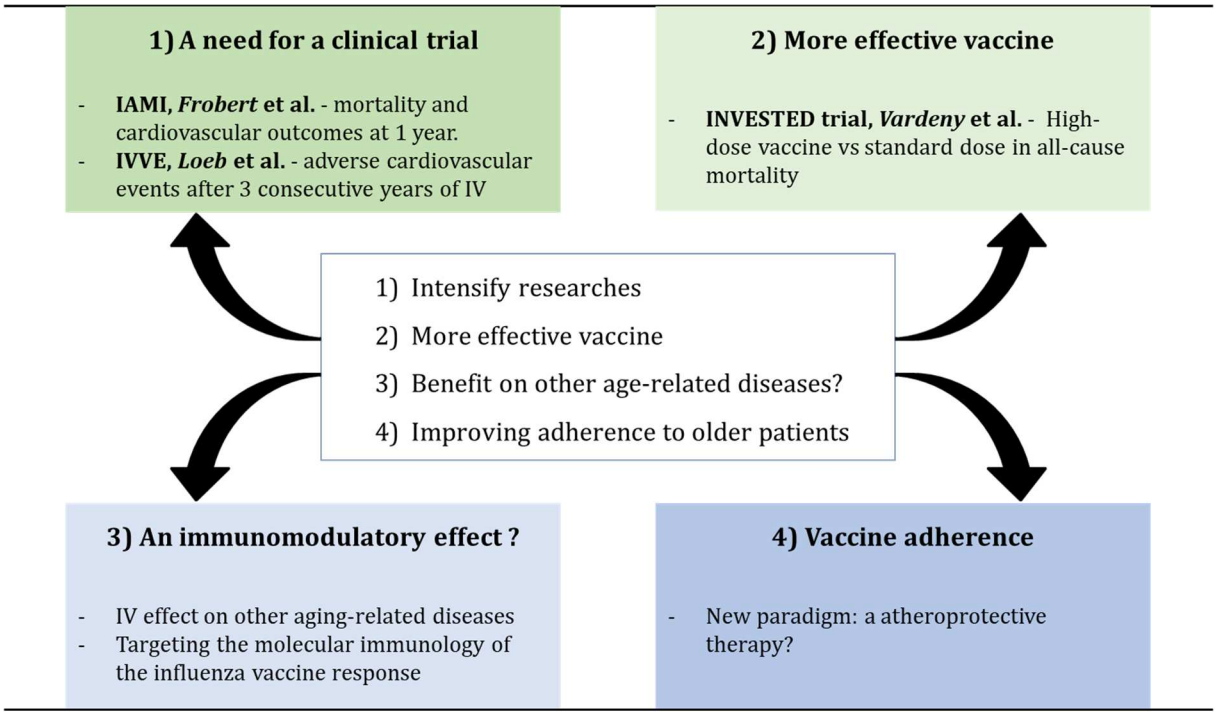

Figure 3 – Future directions

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.

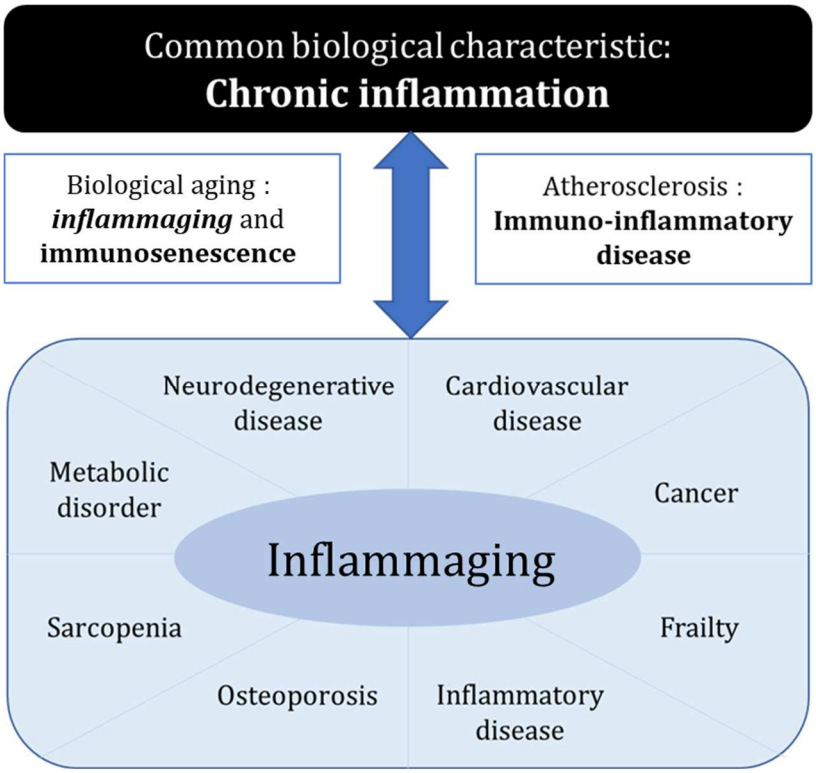

Figure 4 - Aging and aging-related diseases

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 **	• 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