Review Article

Immunosenescence and nutrition: reviewing clinical evidence on pre-, pro- and synbiotics in aging

Renata Ramalho[1,2,3*]

[1]Instituto Superior de Ciências da Saúde Egas Moniz [ISCSEM] – Department of Nutrition, Campus Universitário Quinta da Granja, 2829 – 511 Monte de Caparica, Portugal

[2]Centro de Investigação Interdiscipilinar Egas Moniz [CiiEM], Campus Universitário Quinta da Granja, 2829 – 511 Monte de Caparica, Portugal

[3]Grupo de Estudos em Nutrição Aplicada [GENA] – ISCSEM, Campus Universitário Quinta da Granja, 2829 – 511 Monte de Caparica, Portugal

Abstract

Aging is considered a complex process, characterized by a general decline in physiological functions, as well as increased morbidity and mortality. Being old, healthy and autonomous for the daily life constitute the hallmark of successful aging. However, dependency and frailty are common to people aged 65 or older. The aging process in accompanied by altered immune responsiveness, both at the adaptive and innate levels, that are correlated to malnutrition and frailty. Immunosenescence is also a contributor for increase susceptibility to infections and to vaccination resistance in the elderly.

The impact of aging in mucosal immunity or mucosal immunosenescence has gained the interest of researchers in recent years. Mucosal immune response is impaired in elderly, probably adding a contribution to reduced vaccination efficacy and increased susceptibility to infection. The impact of aging on mucosal immunity may indicate a possible role of therapeutic modulation of mucosal immunosenescence by pre-, probiotics and synbiotics.

In this review, the author gathered the most relevant clinical evidence regarding the effect of pre-, pro- and synbiotics in innate and adaptive immune responses of people aged 65 or older. The concepts and immunological features of immunosenescence and mucosal immunosenescence were also carefully reviewed.

Introduction

World population has been aging in last decades, with profound modifications in demographics and public health [1]. In 2010, 8% of world's population was estimated to be aged 65 or older, corresponding to nearly 524 million people [1]. Triplication of this number is expected by the year 2050, with 1.5 billion people considered old [1]. Some of this dramatic change in world's demography may be explained by the considerable improvements in public health, mainly those leading to a shift in the cause of disease and mortality [2,3]. It is widely accepted that the major health threats early in 20th century, were infectious and parasitic diseases [4,5]. Currently, humanity face a new paradigm in health and disease, being forced to fight against chronic diseases that drag for long periods, causing essentially morbidity and loss of life quality [6-9].

Aging is considered a complex process, characterized by a general decline in physiological functions, as well as increased morbidity and mortality [1,9]. The most important aspect of aging is the chronic inflammatory status, named "inflamm-ageing" [10,11] strictly associated with the deterioration of the immune function, termed "immunosenescence" [12]. Both are causes of increased susceptibility of elderly to infectious diseases, cancer, dementia, cardiovascular diseases and autoimmunity, and of a decreased response to vaccination [10, 13-15]. Research in this field as captured the attention of both researchers and academics, and the role of nutrition in this has gathered more relevance in recent years [16].

In this review, main concepts of immunosenescence and its effect on metabolism were revised. Also, clinical evidence on the effects of pre- and probiotics in aging was gathered and discussed, in a perspective of achieving a successful aging.

Successful aging

Longevity is not always synonymous of many years lived with health and autonomy; in fact, successful aging is a better measure of this [17-19]. Being old, healthy and autonomous for the daily life constitute the hallmark of successful aging [17-19]. It is recognized that the aging process is always somehow accompanied by some degree of physical and metabolic deterioration; but the balance between these expected alterations and the environment and life style may be the answer to achieve a longer life with independence and well-being.

Some regions of the Earth, known as "blue zones", are considered geographic clusters of longevity [20]. The proportion of centenarians and those aged 90 or older in the total population, is consistently increased in these regions; and people not only live longer as they are active past the age of 100 years [20,21]. Examples of "blue zones" are Sardinia in Italy, Okinawa in Japan, Loma Linda in California and Nicoya Peninsula in Costa Rica [20]. The successful aging achieved in these regions may be influenced by environmental factors, such as special local soil, water, and air, a "Mediterranean" dietary pattern, and special psychologic and sociologic conditions [less stress, commensalism, mutual aid], that protects people from the general

decline in physiological functions and maintains independence and physical activity [22]. Studies with centenarians, considered examples of successful aging, have been promising in increasing knowledge about factors that influence senescence and in outline strategies to fight chronic diseases and dependency in older people [21,22].

Immunosenescence

The term immunosenescence was first coined by Roy Walford in 1969 [23]. At this time, autoimmunity was pointed as the core characteristic of immunosenescence. Since then, the concept has been evolving and, nowadays, immunosenescence is regarded as the state of altered immune responsiveness, both at the adaptive and innate levels, expected to arise as aging occurs [12,22,24]. From the perspective of the first line of defence, the process of aging affects both the physical and cellular barriers of the immune system [12,25,26]. It is well accepted that decrease of skin cell replacement, sweat production and overall reduction of barrier function with deterioration of epidermal immune response, occurs [25].

The effect of aging on cells of innate system has been observed in dendritic cells, neutrophils, natural killer cells, macrophages and microglial cells. This effect has been explored in translational studies that point to significant reductions on chemotaxis and phagocytosis, antigen presentation, cytotoxicity, activation of transduction signalling, and secretion of and response to cytokines [27-31]. Regarding cytokines, studies indicated that immunosenescent cells have lower secretion of interferon [particularly, INF-γ] and TNF-α, and have reduced response to IL-2 [32]. In the adaptive arm of the immune system, the main effects of aging have been described as age-related decrease of the novo generation of both B and T lymphocytes [33]. This altered immune response may also be regarded as the decline of the immune response and/or the inadequate elevation of inflammation.

So, this paradoxical context, where chronic low-level inflammation is present along with immunodepression and autoimmunity, has been explained by some central theories: autoimmune, deregulation, immune deficiency, and oxidative theories [10,34,35]. Regardless of the main features explored by those theories, inflammation and oxidative stress have been drawn increasing attention [10,24]. as it has been implicated in many chronic disabling and common diseases that have a clear association with advancing aging [atherosclerosis, type 2 diabetes, osteoporosis, for example].

The impact of aging on immunity

Immunosenescence and innate immunity

As previously mentioned, neutrophils, dendritic cells, monocytes/macrophages and natural killer cells, are the cells of the innate immune system most affected by aging.

The majority of evidence points to the lack of changes in the number of neutrophils, and to a reduction in its physiologic function, although there is still some controversy on this topic [36]. It has been demonstrated that neutrophils functions are severely compromised with age, mainly those related to chemotaxis, phagocytosis, and intracellular killing through radical production [36-40]. There is also evidence that homeostasis of these cells is altered, especially their susceptibility to spontaneous and induced apoptosis which is increased. These alterations in functionality seem to be accompanied by changes in signalling molecules traffic and in signalling of TLRs [38,40].

Regarding monocytes, there is some evidence of decrease in TLR1/TLR2-mediated cytokine production, reduction in CD16- and increase

of in CD16+ cells, with age [41-43]. Number of macrophages seems to be preserved in older people, although some important functions were revealed to be reduced in healthy aging. Some of these functions are phagocytosis, chemo taxis, superoxide production, signal transduction, apoptosis, TLR expression and function, MHC II expression, and cytokine production [41-43]. On the other hand, PGE production was demonstrated to be increase by macrophages with age [41-43].

As well as for other cells of the innate immune system, dendritic cells are also affected by effects of aging process [41]. Both the plasmocytoid and myeloid phenotypes of these antigen presenting cells, of major importance in linking the innate and the adaptive arms of immune system, are compromised in the elderly [44-46]. pDC from older individuals show lower production of IFN I/III and lower capacity for antigen presentation. In addition, mDC, display reduced TLR-mediated signalling, antigen presentation, chemo taxis and endocytosis [36,45].

Important effects of aging on NK cells have been described, both in number, function and phenotype. The absolute number of NK cells is increased with aging, accompanied by a reduction in CD56bright and increased in CD56dim [47,48]. Increased expression of CD57 by Cd56dim cells has also been reposted [47, 48]. These changes may, in part, explain the alterations observed in the phenotype and in the functionality of NK cells during aging, as well as the decrease proliferative response observed in older people. Overall cytotoxicity seems to be unchanged; but when considering the cytotoxicity to the K562 target cells per NK cell, this has been demonstrated to be reduced. Another important alteration observed in aged NK cells is on natural cytotoxicity receptors, mainly the reduction in the expression of the activating receptor NKp30 and expression of NKp46 [47-51]. This may lead to a decreased capacity of these cells to collaborate in the initiation of the adaptive immune response against virus-infected or tumour cells [47-51]. Regarding cytokine production, CD56bright cells of elderly presents increase production of INF-γ, possibly as a compensatory mechanism to maintain immunoregulation in older people, and lower production of chemokines in response to IL-2 or IL-12 [47-51].

Immunosenescence and adaptive immunity

Increased susceptibility to infection and decreased response to vaccination have been two of major concerns in older people [13]. In fact, decreased ability of the elderly to respond to new antigens and vaccinations, contribute to reduced control of infectious diseases later in life, and are a consequence of a senescent adaptive immune system [27, 52].

Impairment of humoral immunity mediated by immunosenescence is less studied than that of cellular immunity [33]. However, it is widely accepted that humoral immune response is compromised by the aging process, especially the functionality and the number of B cells [25]. Data on alterations in B cells point to decreased number of circulating B cells, lower antibodies production, sifts in the magnitude of B cell compartments, changed of specificity repertoires and of B cell dynamics, and overall weakened humoral responses in older humans [53-55]. Both percentage and absolute number of total CD19+ B cells are reduced, and number of B cells precursors in bone marrow seems to be also lower during aging process [56-58]. Regarding functionality, antibodies are less protective, and demonstrate lower ability to opsonize in vitro after vaccination, tan those from young humans [54, 59-61]. Response to influenza after vaccination and recirculating of long-lived antibody plasma cells in the bone marrow, are also reduced in elderly [58]. Interestingly, serum IgG and IgA levels of elderly and centenarians are increased, although the number of peripheral B cells

and their ability to produce antibodies are decreased [62-64]. This paradox is also accompanied by reduction in IgM and unchanging in IgD serum levels [53, 58].

Immunosenescence of T cells is well studied. Evidence from animal and human studies point to reduced percentage and absolute number of circulating CD3+ T cell and of subsets CD4+ and CD8+ [65-67]. A gradual shift from naïve CD45RA+ to activated or memory CD45RO+ cells has been reported, probably explained by thymic involution and the associated differentiation into antigen-experienced memory or effector cells [68]. One important feature observed during aging, is the increased proportion of senescent Cd8+CD28- T cells [68]. This has been correlated with frailty and impairment of vaccination response to influenza in elderly [15, 65]. These senescent CD8+CD28- T cells are dysfunctional and present additional defects on CD40L [CD156+] expression, leading to lower ability to help B cells to proliferate and to produce immunoglobulins [67, 69-71]. Some degree of controversy still remains when considering the effect of aging in CD4+:CD8+ ratio [72].

Mucosal immunosenescence

As previously presented, the effects of aging on both the innate and adaptive immune system seem to increase the susceptibility of older people to infections and to vaccination resistance. Recently, the effects of the aging process on mucosal immune system competence has gathered more interest from researchers and academics [73]. The concept of mucosal immunosenescence has been evolved, and regardless of some controversies there is more information about the impact of aging process on mucosal immune system [73-75]. Studies have been shown that mucosal immune response is impaired in elderly, probably adding a contribution to reduced vaccination efficacy and increased susceptibility to infection [75-77]. This impairment is observed for all the compartments of mucosal immune system, but especially in gut-associated lymphoid tissue [GALT] [77, 78].

Regarding GALT, the most relevant and interesting impairment observed in elderly is related to the composition of microbiota [79]. Recent literature supports that pathogenic bacteria are increased in GALT from elderly, whereas beneficial anaerobes [*Bacteroides* and *Bifidobacteria*] are reduced [80-83]. In fact, bacteria from the genera *Fusobacteria*, *Propionobacteria*, *Clostridia*, *Enterobacteria*, *Streptococci*, *Staphylococci* and yeasts, are demonstrated to be increased in the elderly [73, 79, 84-87]. This shift in intestinal microflora composition has been associated to increased putrefaction, inflammation, and susceptibility to infection [73, 87]. Another element of this equation is the age-associated malnutrition [84, 88, 89]. In fact, the elderly are more susceptible to malnutrition and to their deleterious effects on immunity, than young ones [88]. Malnutrition observed in older people is an important contributor to changes in intestinal microflora composition [82, 90-93]. Some functional alterations resulted from the aging process affect local dendritic cells, especially reducing the uptake of luminal antigens. M cells on Peyer's patches are also affected, presenting lower maturity [94-96]. However, no changes in the number of dendritic cells and Peyer's patches are described in elderly. Regarding secretory IgA, studies have been reported conflicting results. Other important effects in resident macrophages, such as reduction in cytokine production from and in antigen presentation capacity, have been also reported [78, 97, 98].

Modulation of immune response by pre- pro- and synbiotics

Since 1907, that Nobel laureate Élie Metchnikoff´s discoveries on the hypothesis that certain bacteria may modulate the aging process resulted from the activity of putrefactive microbes producing toxic substances in the large bowel, have been gained substantial improvement [99]. The original observations of Metchnikoff that certain rural populations in Europe had exceptionally longer life, living mainly on milk fermented by lactic-acid bacteria, gave rise to the concept of "intestinal autointoxication", which was associated to some physical changes associated with old age, and that seemed to be reverted by the colonization of harmless lactic-acid bacteria and suppression of proteolytic bacteria [99-104]. During the 20th century the interest on probiotics reached high levels, being studied for utility in several distinct conditions. Meanwhile, some disappointing results have cooled the minds of the scientific community on probiotics. In the 21st century, it seems that probiotics are regained attention from the several stakeholders involved in the health process. Although many strains are used as probiotics, the main used in research are *Lactobacillus rhamnosus, Lactobacillus reuteri, Bifidobacteria spp.,* certain strains of *Lactobacillus casei, Lactobacillus acidophilus-group,* and the yeast *Saccharomyces boulardii* [100].

Immunomodulatory effects of probiotics are known to be strain- and dose-dependent [102, 103]. In fact, results are somehow conflicting when attempting to gather the clinical evidence of these food products. However, anti-inflammatory activity of *L. helveticus, L. rhamnosus GG, L. reuteri* and *Bifidobacteria spp.*, had been demonstrated [102]. Some mechanisms of action on immune system, claimed for probiotics are activation of macrophages, NK cells, antigen-specific cytotoxic T lymphocytes, release of anti-inflammatory cytokines, and increase IgA levels [103]. When considering the effects on microbiota, probiotics seem to be able to colonize gut mucosa, competing with pathogenic bacteria for implantation and nutrients [82, 105].

Regarding prebiotics, they are considered to be non-digestible fibres that affect the host's health by selectively stimulating growth and/or activity of some genera of probiotics [such as *Lactobacilli spp.* and *Bifidobacteria spp.*] [100]. These products should be resistant to the actions of stomach acids, bile salts and hydrolysing enzymes in the intestine; and be easily fermentable by the beneficial intestinal microbiota [106]. Prebiotics should also have proved beneficial effects on health of the host. Nowadays, inulin, oligofructose and galacto-oligossacharides are considered prebiotics meeting the FAO/WHO criteria [100].

Synbiotics are a combination of probiotics and prebiotics and represent an interesting form of nutritional modulation [102]. These food products may increase the chance of survival of probiotics in the gut, and therefore enhancing their growth and activity [100].

Pre- pro- and synbiotics in aging immune system

When considering the effects of aging in immune system, and associating the possible effects of pre-, pro- and synbiotics in modulating immune responses systemically and in local immune system, it may be expected that probiotics exert some beneficial effects for elderly [101,104,106,107]. The question is whether the mechanistic effects studied in cell cultures and animal models, effectively applies to humans, and what kind of recommendation may be done. Some evidence is available concerning randomized clinical trials [RCT] addressing this issue [108-117] . This has been gathered, and is presented in table 1. The major problem when gathering these studies is the considerable amount of heterogeneity which difficult a metanalysis, and therefore a convincing recommendation. As it will be discussed later, this heterogeneity is mainly due to different dosages, strains, duration of treatments and formulations tested in clinical trials.

Probiotics

All the six RCT [108-113] included in this review demonstrated some degree of positive effects in innate, adaptive and/or mucosal immunity of people aged 65 or older, with probiotics ingestion. These effects are summarized in table 1. Enhancement of NK cell activity was demonstrated in four studies [108,109,111,112], anti-inflammatory effects in four [108-110,113], and increased tumoricidal and/or phagocytic activity in two [111,113]. Regarding effects on adaptive immune system, increased number of T cells [total CD3+ and/or CD4+

naïve and/or CD8+ naïve] was described in two studies [109,112]. In the study from Ouwehand *et al.* increased number of beneficial bacteria and reduction of pathogenic bacteria were observed in faeces, after six-month consumption of oat-based drink/day *B. longum* [110]. As demonstrated in table 1, interventions are considerable different between studies, making it difficult to compare results between them.

Prebiotics

Two RCT addressed the effect of prebiotics in immune system of elderly [114,115]. One tested a mixture of galacto-oligosaccharide

Table 1 Characteristics and main results of randomized controlled trials addressing the effect of pre-, pro-, and synbiotics in systemic or mucosal immune systems of older humans.

Study / Reference	Design and Participants	Intervention	Outcomes	Results
Probiotics				
Dong *et al.*, 2013 [108]	• Randomized, placebo-controlled, single-blinded crossover study • 30 healthy volunteers (18 females), 54-74 years old.	• 65 ml/daily Yakult light (*L. casei* Shirota 6.5x10⁹ CFU/bottle) twice a day for 4 weeks. • Placebo: 130 ml skimmed milk twice a day for 4 weeks	• Biochemical profiles • Inflammatory markers • Innate immunity • Adaptive immunity	• Increase in NK cell activity, reduction of MFI of CD25 expression on resting T cells. • Trend towards an increased ratio IL-10:IL-12.
Moro-Garcia *et al.*, 2013 [109]	• Multi-centre, randomized, placebo-controlled, double-blinded study • 47 healthy volunteers (40 females), 65-90 years-old	• 3 capsules/day *L. delbrueckii* subsp *bulgaricus* 8481 (3x10⁷ CFU) + *S. thermophilus* for 6 months • Placebo: 3 capsules/day with cornstarch for 6 months	• Biochemical profiles • Inflammatory markers • Innate immunity • Adaptive immunity	• Increase in NK cell percentage. • Increase in naïve CD4+ and naïve CD8+ T cells. • Decrease in IL-8. • Increase in hBD-2.
Ouwehand *et al.*, 2008 [110]	• Randomized, placebo-controlled, double-blinded study • 209 elderly in nursing homes (170 females), 84.3±0.98 years old	• 1 bottle oat-based drink/day *B. longum* 2C+ *B. longum* 46 for 6 months • Placebo: 1 bottle oat-based drink/day for 6 months	• Faecal microbiota • Inflammatory markers	• Increase in faecal levels of *B. adolescentis* and *B. catenulatum* • Modest decrease in IL-10.
Gill *et al.*, 2001[111]	• Randomized, controlled, single-blinded crossover study • 27 healthy volunteers (16 females), 60-84 years-old	• Group A: 250ml/day reconstituted milk with *L. rhamnosus* HN001 (5x10⁹ CFU) for 3 weeks • Group B: 250ml/day reconstituted milk with *L. lactis* HN019 (5x10⁹ CFU) for 3 weeks • Control: 250ml/day non-supplemented milk for 3 weeks	• NK cell activity	• Increase in CD56+ (NK cells) for both groups A and B • *In vitro* tumoricidal activity of PBMC for both groups A and B
Gill *et al.*, 2001 [112]	• Randomized, controlled, single-blinded crossover study • 30 healthy volunteers (18 females), 63-84 years-old	• Group high-dose: 250ml/day reconstituted milk with *B. lactis* HN019 (5x10¹⁰ CFU) for 2 weeks • Group low-dose: 250ml/day reconstituted milk with *B. lactis* HN019 (5x10⁹ CFU) for 2 weeks • Control: 250ml/day non-supplemented milk for 2 weeks	• Innate immunity • Adaptive immunity	• Increase in total CD3+ T cells, CD4+ T cells, CD25+ T cells and CD56+ (NK cells) for both high-dose and low-dose groups, with no differences between them. • *In vitro* phagocytic activity of PMN and MNN for both high-dose and low-dose groups, with no differences between them.
Arunachalam *et al.*, 2000 [113]	• Randomized, placebo-controlled, double-blinded study • 25 healthy volunteers (18 females), 60-83 years-old	• 180ml reconstituted milk with *B. lactis* HN019 (1.5x10¹¹ CFU) twice a day for 6 weeks • Placebo: 180 ml reconstituted milk twice a day for 6 weeks	• Innate immunity	• Increase in IFN-γ production • Enhance in phagocytic capacity of PMN
Prebiotics				
Vulevic *et al.*, 2015 [114]	• Randomized, placebo-controlled, double-blinded cross-over study • 40 healthy volunteers (25 females), 65-80 years-old	• 5.5g/day B-GOS (galacto-oligosaccharide mixture) for 10 weeks • Placebo: 5.5g/day Maltodextrin for 10 weeks	• Faecal microbiota • Inflammatory markers • Innate immunity	• Increase in faecal number of bifidobacteria and bacteroides. • Increase in IL-10 and IL-8 production. • Reduction in IL-1β production • Increase in NK cell activity.
Bunout *et al.*, 2002 [115]	• Randomized, placebo-controlled, double-blinded study • 43 healthy volunteers, ≥70 years	• 6g/day 70% raftilose+30% raftiline mixture for 28 weeks • 6g/day Maltodextrin for 28 weeks	• Innate immunity • Adaptive immunity	• No effect.
Synbiotics				
Macfarlane *et al.*, 2013 [116]	• Randomized, placebo-controlled, double-blinded cross-over study • 43 volunteers (22 females), 65-83 years-old	• 1 gelatin capsule *B. longum* (2x10¹¹ CFU)+6g Synergy I (Inulin+Oligofructose) twice a day for 2 weeks • Placebo: 1 capsule potato starch+6g Maltodextrose twice a day for 2 weeks	• Faecal microbiota • Inflammatory markers • Biochemical profiles	• Increase in number of faecal bifidobacteria (*Actinobacteria* and *Firmicutes*). • Reduction in faecal *Proteobacteria*. • Reduction in TNF-α.
Ouwehand *et al.*, 2009 [117]	• Randomized, placebo-controlled, double-blinded parallel study • 47 volunteers (35 females), > 65 years-old	• 1 sachet (5-5.5g) *L. acidophilus* NCFM (2x10⁹ CFU)+Lactitol twice a day for 2 weeks • Placebo: 1 sachet (5g) Sucrose twice a day for 2 weeks	• Faecal microbiota • Mucosal immunity • SCFA production	• Increase in number of faecal *Bifidobacterium* • Increase in spermidine levels • Modest increase in PGE2 concentration in faeces

[GOS] [114], and the other, a mixture of raftiline and raftilose [115]. The study from Bunout *et al.*, failed to demonstrated effects of tested intervention [115], but in the study from Vulvelic *et al.*, increased number of beneficial bacteria in faeces, anti-inflammatory action through reduction of IL-10 and IL-1 and increased NK cell activity, were demonstrated [114].

Synbiotics

Two RCT studied synbiotics in this context [110,116], one testing a combination of *B. longum*, inulin and oligofructose [116] and the other, L. acidophilus NCFM and lactilol [117]. Both studies demonstrated increased number of beneficial bacteria in faeces [table 1]. In the study from Macfarlane *et al.* [116], it was also demonstrated reduction of TNF-α levels, and in the study from Ouwehand *et al.*, reduction of spermidine levels [117].

Conclusion

Senescence of the immune system represents a challenge for dealing with increased prevalence of chronic diseases in an increasingly aging population. Immunosenescence affects both the innate and adaptive immunity, correlating with malnutrition and frailty, common to people aged 65 or older. In recent years, mucosal immunosenescence has gained the interest of researchers. The impact of aging on mucosal immunity, especial on microbiota, may indicate a possible role of therapeutic modulation of mucosal immunosenescence by pre-, probiotics and synbiotics. Gathering evidence on RCT addressing the effects of pre-, probiotics and synbiotics in immunosenescence outcomes in elderly, demonstrated that the evidence is scarce, with reduced number of studies and including small groups of participants. Also, the heterogeneity in designs and types of interventions is an important limitation to compare results and to perform a recommendation. Despite this limitation, it seems that pre-, probiotics and synbiotics may represent a nutritional modulation for immunosenescence. Larger, well-designed, RCT, with lower heterogeneity in interventions, are need to prove the interest of these food products in contributing to a healthy immune system through aging, and therefore an option to achieve successful aging.

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Conflict of interest

None to declare.

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