

Inflammation and chronic disease

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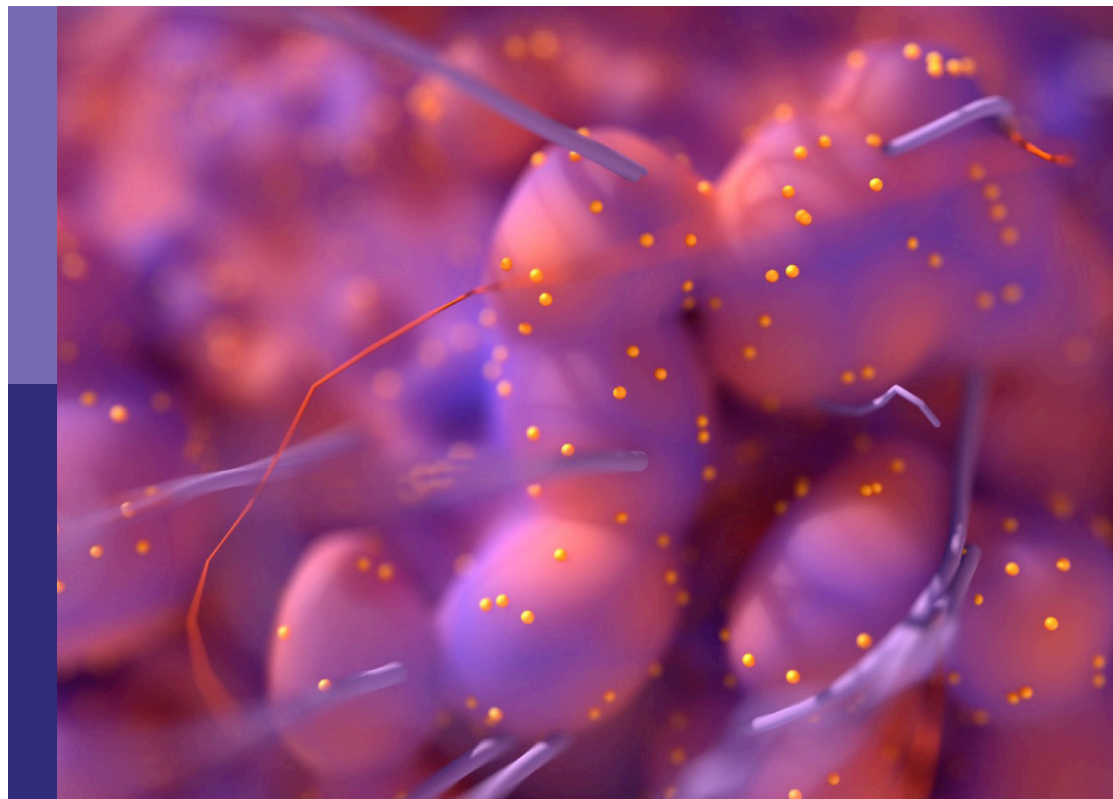
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Inflammation and chronic disease

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Editorial: Inflammation and chronic disease

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Editorial on the Research Topic Inflammation and chronic disease

Introduction

Inflammation is directly associated with the morbidity and mortality of a diverse number of chronic health conditions including cardiovascular disease (1–3), chronic kidney disease (4), diabetes mellitus (5, 6), non-alcoholic fatty liver disease (7), cancer (8, 9), autoimmune diseases (10, 11), and neurodegenerative (12) and behavioral health disorders (13) (Figure 1). Not only is inflammation the byproduct of chronic disease, it also has a mechanistic role in the underlying etiology and pharmacoprevention of diseases such as atherosclerosis (14). For example, some of the most common mutations in age-related, clonal hematopoiesis of indeterminate potential (CHIP) increase the expression of inflammatory genes, potentially explaining why CHIP is associated with almost twice the risk of coronary artery disease (15–17).

Various acute and chronic factors can modulate inflammation, including infection, the social and physical environments (18), lifestyle (19–21), diet (22, 23), and physical activity (24–27) (Figure 1). A PubMed search of “chronic inflammation” leads to over 170,000 results, and stalwart medical institutions propose diets targeting chronic inflammation (28, 29). Moreover, translating such knowledge to disease therapy has improved outcomes, such as using exercise to reduce inflammation in patients with depression (30), COPD (31), and frailty (32). Despite this, there is a lack of anti-inflammatory guidelines to prevent and treat chronic disease from bench to practice, and a deeper understanding of the complex relationship between inflammation and chronic disease development and progression is needed.

Heart disease and cardiometabolic disease

A body of experimental evidence demonstrates how interferons (IFNs) and IFN-related pathways play important roles both in the inflammation commonly associated with heart disease pathogenesis as well as in the protection against heart disease (Tran et al.). While it is unlikely that measuring a single plasma IFN will be of prognostic significance in managing heart disease, immense advances in single cell technologies are helping elucidate the molecular mechanism of heart disease. Therapeutic, immunosuppressive strategies to reduce IFN or IFN-related pathway signaling come with an increased malignancy and infection risk, and targeting downstream pathways, such as cyclic GMP-AMP synthase-stimulator of interferon genes signaling,

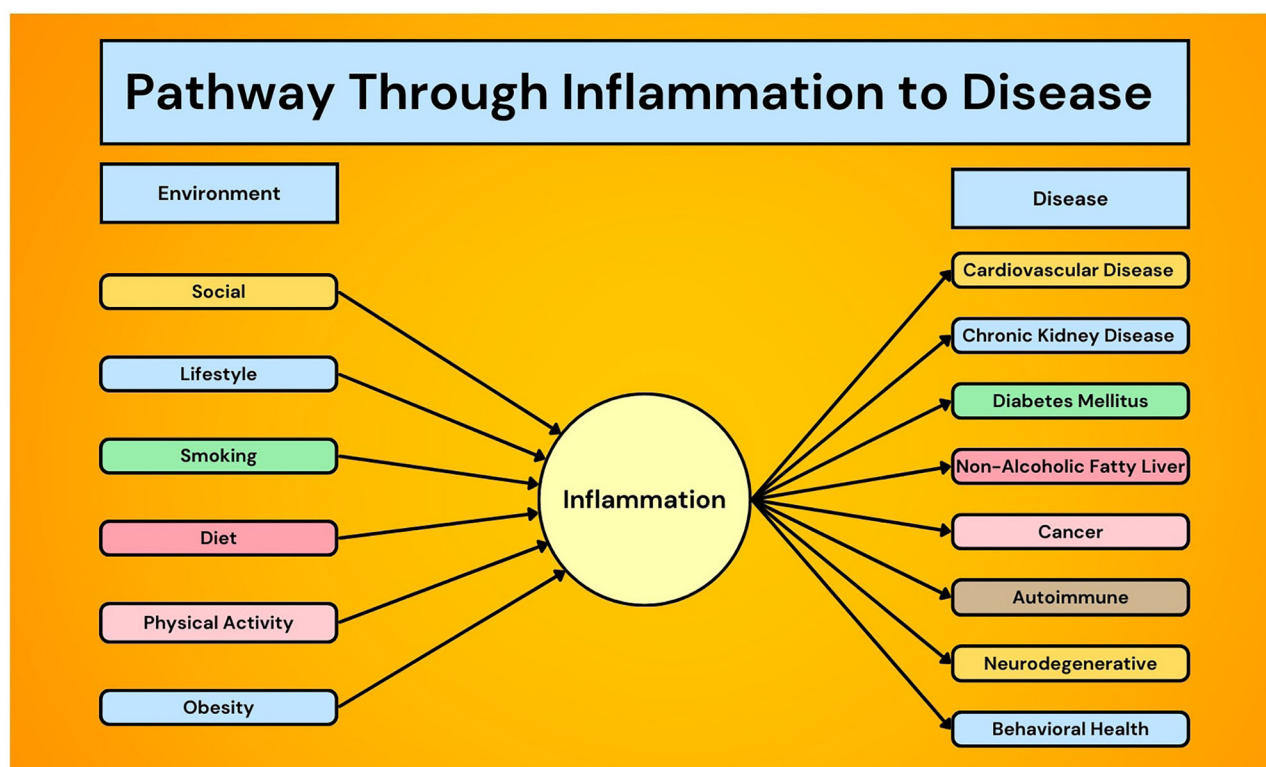


FIGURE 1
Unifying theory of inflammation and chronic disease prevention.

may theoretically overcome these side effects by allowing other immune defense pathways to remain intact (Tran et al.). When the advanced lung cancer inflammation index (ALI) was used to assess inflammation in hypertensive patients, it determined that lower levels of inflammation (i.e., higher ALI) were associated with reduced risk of cardiovascular death (Tu et al.). However, because of the way ALI uses body mass index (BMI) in its equation and how high BMI is an established risk factor for cardiovascular death, it is recommended that ALI not be used as a prognostic marker for cardiovascular death in hypertensive patients with BMI ≥ 35.5 kg/m².

US adults with undiagnosed cardiometabolic disease have a higher risk of elevated HsCRP (Mainous, Sharma et al.). Furthermore, risk of the metabolic disorder nonalcoholic fatty liver disease (NAFLD) was linearly associated with the inflammation-related biomarkers SII, neutrophil-to-lymphocyte ratio (NLR), and lymphocyte-to-monocyte ratio (LMR) and non-linearly associated with platelet-to-lymphocyte ratio (PLR) when natural logarithm (ln) transformed (Liu et al.). These results further elucidate inflammation's clinical significance in NAFLD may assist with ongoing research to improve diagnosis and treatment options.

Cancer and immunoinflammatory dermatoses

Levels of vascular adhesion protein-1 (VAP-1), a dual-function glycoprotein with an important role in inflammation

and tumor progression, was associated with an increased 12-year risk of cancer incidence, cancer mortality, and all-cause mortality in a Taiwanese population, a predictive performance that was better than smoking (Chen et al.). Two inflammation-related biomarkers, systemic immune-inflammation index (SII) and product of platelet count and neutrophil count (PPN), were independent risk factors for kidney cancer incidence and may aid in the development of targeted screening strategies for at-risk patients (He et al.). Inflammasomes, immune-functional protein multimers that are closely linked to the host defense mechanism and can activate various inflammatory signaling pathways closely associated with malignancies, have become a novel target of more than 50 natural extracts and synthetic small molecule agents as prospective therapies for common cancers (Gu et al.). Another novel inflammatory target for cancer therapeutics are neutrophil extracellular traps (NETs), web-like structures containing DNA and released from the nucleus or mitochondria (Zhong et al.). NETs, important structures in innate immunity and the progression of inflammatory diseases, are being investigated for their role in potentially treating multiple cancers, especially metastatic cancer.

Circulating inflammatory cytokines' role in the development of immunoinflammatory dermatoses offers new prevention and therapeutic targets. In a Mendelian randomization study, both IL-4 and IL-1RA may have inhibitory functions in atopic dermatitis pathogenesis (Li et al.). Conversely, IL-4 and SCGF-b may have promoting functions in the pathogenesis of vitiligo and psoriasis, respectively.

Social environment, diet, and physical activity

A considerable proportion of US adults have elevated inflammation as measured by highly sensitive C-reactive protein (HsCRP), especially minorities and individuals with low socioeconomic status (Mainous, Orlando et al.). While either inflammation or poverty alone each confer about a 50% increased risk in all-cause mortality in US adults aged 40 and older, individuals with both inflammation and poverty have a 127% increased heart disease mortality risk and a 196% increased cancer mortality risk, revealing that the combined effect of inflammation and poverty on mortality is synergistic in this population (Mainous, Orlando et al.). Therefore, both systemic inflammation and poverty could become a focus of primary care for preventing disease and monitoring its progression.

Low-grade chronic inflammation can be initiated and aggravated by specific key dietary factors, particularly sugars and mixed processed foods, the consumption of which has significantly increased over the past 30 years (Ma et al.). The negative impact that a high-sugar diet has on certain autoimmune conditions, such as rheumatoid arthritis, multiple sclerosis, psoriasis, inflammatory bowel disease, has been mechanistically linked to its pro-inflammatory effects (Ma et al.). For example, obesity has been strongly associated with low-grade chronic inflammation, but in the case of psoriasis, new research suggests that dietary sugars and fats mediate the inflammatory stimulation of psoriasis rather than obesity itself (Ma et al.). Similarly, physical activity influences inflammation with connections to disease severity. For example, physical activity following surgical resection for colon cancer is associated with a significantly increased disease-free survival, and inflammation has been hypothesized to be the linking factor (Brown et al.). Even though aerobic exercise was not associated with dose-response reductions in HsCRP or IL-6 in a randomized, dose-response trial of 39 stage I-III colon cancer survivors, cancer stage modified the association (Brown et al.). Specifically, exercise was not associated with inflammatory biomarkers in stage I-II disease, and 300 min/week of moderate-intensity aerobic exercise (high-dose) was not associated with inflammation in stage III disease, but 150 min/week of moderate-intensity aerobic exercise (low-dose) did reduce HsCRP and IL-6 in stage III disease (Brown et al.). However, the biological reason why cancer stage modifies the association between exercise dose and inflammatory biomarker levels remains unclear and is being prospectively studied in an ongoing randomized trial of exercise in colorectal cancer survivors (NCT03975491).

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Conclusion

Inflammation has an established connection with the etiology and progression of numerous major chronic diseases, but no specific guidelines exist for clinicians to use inflammatory markers to guide prevention, diagnosis, or treatment. Equally as important, the opportunity to discover a breakthrough treatment for such common chronic diseases may be right at our fingertips with the mechanistic knowledge of inflammation's role in disease pathogenesis. Future large, prospective clinical trials are needed to further elucidate the findings of mechanistic and observational trials and translate them to the bedside.

Author contributions

FO: Conceptualization, Investigation, Writing – original draft. AM: Conceptualization, Supervision, Writing – review & editing.

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Excessive intake of sugar: An accomplice of inflammation

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High sugar intake has long been recognized as a potential environmental risk factor for increased incidence of many non-communicable diseases, including obesity, cardiovascular disease, metabolic syndrome, and type 2 diabetes (T2D). Dietary sugars are mainly hexoses, including glucose, fructose, sucrose and High Fructose Corn Syrup (HFCS). These sugars are primarily absorbed in the gut as fructose and glucose. The consumption of high sugar beverages and processed foods has increased significantly over the past 30 years. Here, we summarize the effects of consuming high levels of dietary hexose on rheumatoid arthritis (RA), multiple sclerosis (MS), psoriasis, inflammatory bowel disease (IBD) and low-grade chronic inflammation. Based on these reported findings, we emphasize that dietary sugars and mixed processed foods may be a key factor leading to the occurrence and aggravation of inflammation. We concluded that by revealing the roles that excessive intake of hexose has on the regulation of human inflammatory diseases are fundamental questions that need to be solved urgently. Moreover, close attention should also be paid to the combination of high glucose-mediated immune imbalance and tumor development, and strive to make substantial contributions to reverse tumor immune escape.

KEYWORDS

macrophages, autoimmune disorders, Th17 cells (Th17), low-grade chronic inflammation, TGF-beta, IL-1beta

Introduction

It is well known that high-sugar consumption is a hallmark of the Western diet (1). Dietary sugars mainly refer to fructose and glucose which are naturally present in fruits and some vegetables (2, 3). Their molecular formula is $C_6H_{12}O_6$ and they are isomers of each other (4). Fructose and glucose are both considered to be sweet sugars, yet fructose is

the sweeter of the two. HFCS is a common sweetener and preservative made from the simple sugars' fructose and glucose. HFCS-55 and HFCS-42, the most commonly utilized form that is used in beverages and baked goods, contains 55% and 42% fructose, respectively, with the remainder of the of the syrup being glucose (5). Since the 1970s, the amount of HFCS has increased in foods that are common within the Western diet (5, 6). The United States currently is the major user of HFCS, but HFCS is now produced throughout the world with factories on every continent except for Antarctica (5, 7). The consumption of these sugars, particularly in sugary soft beverages (SSB), became a major contributor to sugar intake, and the relationship between SSB and cardiometabolic diseases reflects the potential effects of fructose and glucose (8, 9). At the beginning of the twenty-first century, the U.S. Department of Agriculture reported that the consumption of soft drinks per capita in the United States had increased by about 500% over the past 50 years (10). To make matters worse, approximately 12% of infants consumed sugary sugar-sweetened beverages, a population who had a higher consumption of confectionaries and lower intake of fruits and vegetables only a couple of years later (10). In Brazil, consumption of sugary soft drinks roughly quadrupled from 1974 to 2003, and in 2009, Brazilian adults consumed about 100 ml/day of SSB (11, 12). In addition, in Europe, sugar consumption in different countries is between 7% and 25% of total energy intake (12). With the deepening of research on the relationship between high sugar diets and human health, the potential threat of high sugar diets to the incidence of noncommunicable diseases has become increasingly recognized (13).

A growing body of research suggests that excessive consumption of processed foods containing dietary sugars or HFCS is strongly linked to the development of obesity (14, 15), T2D (16, 17), metabolic syndrome (16) and cardiovascular disease (18). In 2004, Bray and his colleagues published a review article in the American Journal of Clinical Nutrition that drew attention to the potential relationship between sugar and obesity (19). This paper analyzed food consumption patterns using USDA food consumption tables from 1967 to 2000, and found that consumption of HFCS significantly outperformed changes in intake of any other food over that time period, ultimately confirming that HFCS consumption in high-calorie sweet drinks played a role in the obesity epidemic (20). As the study of sugar and obesity continues to deepen, researchers are looking at whether simple sugars, such as glucose and fructose, contribute to obesity. Some comprehensive information suggests that while both fructose and glucose contribute to weight gain (21), fructose intake is more likely to promote lipid deposition in visceral adipose tissue (VAT), while glucose consumption appears to favor subcutaneous adipose tissue (SAT) deposition (3). Other studies have shown that fructose intake appears to increase triglyceride concentrations in healthy male and decrease glucose tolerance and insulin

sensitivity in obese older adults, compared with an equal-calorie glucose diet (3, 22). However, both intracellular triglyceride levels and insulin metabolism are associated with diabetes. Increased fructose or glucose intake is known to indicate a higher risk of T2D in adults, but the pathogenesis of the two is different (17). Glucose mediates the development of T2D through its high glycemic index, leading to interruption of insulin secretion (17). Fructose on the other hand is associated with a variety of factors, including weight gain, influence on insulin sensitivity, and fatty acid synthesis (23, 24). In addition, SSB made with HFCS can increase the risk of T2D by affecting blood sugar metabolism (25).

Another meta-analysis, which collected prospective cohort studies of 1 year or longer using a first-order linear mixed effects model, found a negative linear dose-response relationship between SSBs and metabolic syndrome (RR 1.14 at 355 mL/d), confirming the association between sugary foods and the onset of metabolic syndrome (26). Relevant randomized controlled trials have also confirmed that people who drink at least one soft drink a day have a 44% higher risk of developing metabolic syndrome than people who do not drink soft drinks (27). Similarly, higher sugar intake is associated with cardiovascular disease. Analysis of The National Health And Nutrition Examination Survey (NHANES) III-related mortality cohort data shows that the intake of added sugar and SSB can lead to the occurrence of hypertension, stroke, coronary heart disease, and dyslipidemia, thereby increasing the risk of death (9, 18). In 1987, Hwang et al. studied Sprague-Dawley rats with fructose in their diet and found for the first time that a high-fructose diet was associated with hypertension (28). Subsequent studies confirmed that the increase in blood pressure caused by a high-fructose diet was due to the activation of the sympathetic nervous system (29, 30). Several other statistical studies conducted follow-up surveys of different populations and concluded that SSB intake is positively correlated with coronary heart disease (31–34), vascular events (35), heart failure (36), and stroke (37), but has nothing to do with subclinical atherosclerosis (38). Therefore, it is necessary to strengthen the social supervision of sugary processed foods. The World Health Organization (WHO) believes that sugars consumption varies with age and country, and their guidelines strongly recommend reducing sugar intake to less than 10% of total energy intake (10). The Scientific Advisory Committee on Nutrition in England (SACN) has also issued a similar policy, recommending that upper limit of sugar intake should not to exceed 5% of total energy intake (19). Despite this, it is difficult for many people of all ages to reduce, let alone eliminate, their intake of sugary drinks (10).

Although the research on the relationship between dietary sugars and the above diseases has been relatively thorough, the impact of these sugars on inflammation was previously unknown. In recent years, as more researchers have explored the relationship between high-sugar diet and inflammation, people have found that

excessive sugar intake is closely associated with the development of low-grade chronic inflammation and autoimmune diseases (Figure 1). Low-grade chronic inflammation has long been linked to obesity and increased body fat, and excess dietary sugar intake is a key contributor to obesity and weight gain. Autoimmune disease is a common disorder caused by the immune system attacking its own normal tissues. Although dietary structure is considered to be a key cause of autoimmune diseases, the impact and mechanism of dietary sugars on it has not been revealed until recently. Based on this, this paper reviews the effects and related regulatory mechanisms of excessive consumption of dietary sugars on inflammatory diseases discovered in recent years. By summarizing the current research progress, it has been revealed that dietary sugar is a key factor in inducing low-grade chronic inflammation, autoimmune diseases, and even neuroinflammation.

Effects of dietary sugars on low-grade chronic inflammation

It has been shown that excessive intake of dietary sugars can cause metabolic disorders and induce the increase of inflammatory mediators and certain pro-inflammatory cytokines in various tissues, which leads to insulin resistance and low-grade chronic inflammation (39, 40). Low-grade chronic inflammation could be caused by factors secreted by adipose tissue, inflammatory factors secreted by liver tissue, and increased intestinal permeability, which may eventually lead to the development of cardiometabolic diseases (39, 41). Therefore, the association between high sugar intake and increased risk of

chronic disease may be mediated in part by low-grade chronic inflammation. In low-grade chronic inflammation, the pro-inflammatory molecules mainly included Toll-like receptor 4 (TLR-4), plasma C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and monocyte chemotactic protein 1 (MCP-1), E-selectin (E-selectin), plasminogen activator inhibitor 1 (PAI-1) as well as others (40, 42, 43). Several randomized trials have investigated the relationship between dietary sugars and systemic inflammation. Faizan et al. distributed beverages containing 50 grams of fructose, glucose, and sucrose to healthy subjects and found that all three increased blood lipid and hs-CRP levels, but fructose and sucrose were significantly more effective than glucose (44). A follow-up prospective trial of six 3-week dietary interventions in 29 healthy young men showed that low to moderate intake of SSBs containing HFCS had potentially harmful effects on low density lipoprotein (LDL) particles, fasting glucose, and hs-CRP (45). However, Jessica and colleagues found that there was no significant change in hs-CRP and IL-6 levels, markers of low-grade chronic inflammation, at the end of the diet period in normal-weight and obese adults who consumed four servings of beverages containing fructose, glucose or HCFS in addition to a standard diet over three eight-day periods. It was concluded that excessive consumption of fructose, HFCS, and glucose from SSBs over 8 days had no difference in low-grade chronic systemic inflammation in normal-weight and obese adults (39). Nor and his team came to similar conclusions. They found no significant differences in inflammatory biomarkers such as CRP, IL-1 β , IL-6, and TNF- α in all dietary groups after 12 weeks in parallel trials of several high-fructose beverages (46). This contradiction

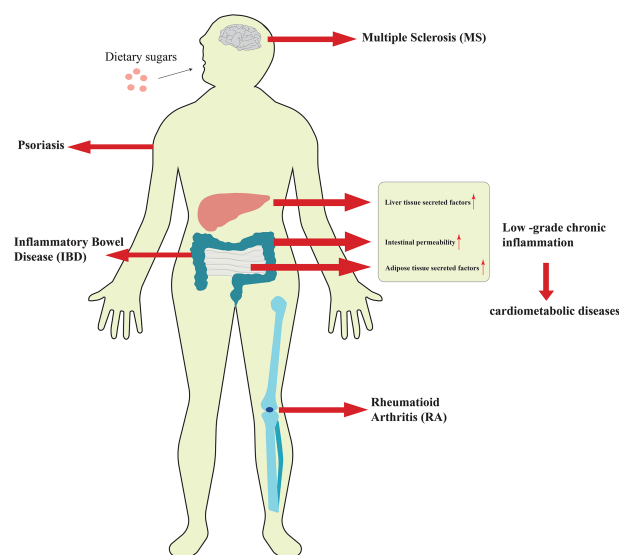


FIGURE 1

Excessive consumption of dietary sugars is closely related to the occurrence and development of inflammation.

may be caused by the age and physical condition of the subjects and the difference in sugar intake. In addition, other studies showed that lipocalin-2, e-selectin, MCP-1 and PAI-1, all markers of systemic inflammation, were also up-regulated in high-fructose fed rats (42, 47).

Adipose tissue is one of the largest endocrine organs in the body and affects local and systemic immune function and metabolism by secreting inflammatory factors (43). Glucocorticoids are the key to the pathogenesis of monosaccharide-induced metabolic syndrome (48). In rats fed a high fructose diet, adipose tissue expressed more corticosterone (CORT), which was then offset by increased levels of macrophage migration inhibitor (MIF) (43, 48). The activity of nuclear factor- κ B (NF- κ B) decreased in adipose tissue, and the expression of inflammatory factor TNF- α did not change. In liver tissue, the level of 11 β HSD1 protein was elevated, but did not affect intracellular CORT levels or downstream glucocorticoid signaling. Therefore, the activation of NF- κ B was enhanced, and the level of pro-inflammatory factor TNF- α was increased (41). This could be interpreted as a tissue-specific result of the regulation of metabolic inflammation by high fructose intake. In another study in rats, fructose reduced fatty acid oxidation by decreasing liver peroxisome-proliferator-activated receptor α (PPAR- α) activity, ultimately leading to increased NF- κ B activity (49). Fructose consumption, on the other hand, can induce liver and systemic inflammation through intestinal changes. It was found that fructose can promote the translocation of microbial substances from the intestinal tract to the portal vein circulation, activate the NF- κ B and JAK2/STAT3 pathways through TLR4, and release inflammatory factors such as IL-1 β , IL-6, and TNF- α (50, 51). At the same time, fructose intake can also increase intestinal permeability and promote the release of inflammatory factors to the liver, thereby increasing liver and systemic inflammation (52). The researchers also found that fructokinase, a key enzyme in fructose metabolism, plays an important role in inflammation caused by non-alcoholic fatty liver disease. Fructokinase knockout mice fed a high-sugar or high-fat diet were protected from liver inflammation and fibrosis, and the expression of inflammatory factors CD68, TNF- α , MCP-1, smooth muscle actin, type I collagen, and TIMP1 was reduced (53). Similarly, liver inflammation and fibrosis also occurred in mouse models with low density lipoprotein (LDL) receptor defects that were fed the Western diet and liquid fructose (54). Another study showed that high fructose consumption can also have damaging effects on the hippocampus, an area of the brain important for learning and memory (55). The role of high fructose in hippocampal inflammation was confirmed by analysis of inhibition of phosphorylation of Ser 307 by hippocampal insulin receptor substrate 1 (IRS-1), protein levels of (NF- κ B), and mRNA levels of related inflammatory factors (56).

Effects of dietary sugars on autoimmune diseases

Autoimmune diseases (AID) are T cell-mediated inflammatory pathologies (57). Normally, the body's immune system does not respond to its own components, known as autoimmune tolerance. AID is an immune pathological state in which the body's autoimmune tolerance mechanism is deregulated or destroyed, resulting in damage or dysfunction of its own tissues and organs (13). The incidence of AID has increased in recent decades, but the reasons for this remain unclear. Current research shows that individual genetic susceptibility and environmental factors are closely related to the disease (58, 59). Although dietary changes, such as high salt intake (60, 61), are thought to be closely associated with increased incidence of AID, the effects and mechanisms of high-sugar diets include rheumatoid arthritis (RA), multiple sclerosis (MS), psoriasis, and inflammatory bowel disease (IBD) have only been uncovered in recent years (13, 57, 62).

Effects of dietary sugars on rheumatoid arthritis

Rheumatoid arthritis (RA) is one of the most common systemic, chronic, autoimmune diseases caused by genetic, environmental, and endogenous factors (63). It is characterized by systemic inflammation and persistent synovitis (64). In recent years, numerous studies have shown that sugar-sweetened beverages play a key role in the pathogenesis of RA (63, 65, 66). In a follow-up survey, researchers found that women who drank \$1 a day of sugar-sweetened beverages had an increased risk of seropositive RA compared with women who didn't drink sugar-sweetened beverages, with a greater risk among women over 55 (64). A subsequent study showed that the reason why sugar-sweetened beverages can cause RA, in addition to their important role in the autoimmune mosaic, is that it is more likely to alter the microbiome, thereby affecting downstream inflammatory pathways (63). High consumption of glucose, fructose, and sugar-sweetened beverages is known to reduce the beneficial flora in the gut, especially *Prevotella*, which has been found to be associated with the pathogenesis of RA (67). In addition, the Mediterranean diet has been shown to reduce the incidence of diseases such as RA compared to a high-sugar Western diet (66, 68).

Effects of dietary sugars on multiple sclerosis

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system with symptoms that affect multiple systems throughout the body, including visual impairment,

movement disorders, fatigue, cognitive and emotional disturbances, pain, and more (69). In MS, immune cells cross the blood-brain barrier (BBB) into the central nervous system to attack self-antigens, resulting in BBB disruption and loss of oligodendrocytes and myelin, leading to axonal degeneration and permanent neurological deficits (69, 70). Many studies have shown that lifestyle choices, including diet, can affect some of the symptoms of MS, and it seems that people with MS can relieve their symptoms by improving their eating habits (70). For example, one study noted that subjects with multiple sclerosis ate more carbohydrates than the control group, but there was no difference in BMI between the two groups. The researchers attributed this to the small sample size used in the study (71). Although the effect of a high-sugar diet on MS has not been confirmed in clinical studies, it has been found that high-glucose and high-sucrose diets can aggravate the disease progression of experimental autoimmune encephalomyelitis (EAE) in a disease model of MS (i.e., the EAE model) (13, 57). Both studies found that high sugar intake increased the proportion of CD4⁺ cells in EAE mice and exacerbated neuroinflammation in the brain and spinal cord, but both studies looked at the deleterious effects of high sugar diets from different pathogenic mechanisms and confirmed two things. On the one hand, high-glucose diet can directly act on CD4⁺ T cells, by inducing T cells to differentiate into Th17 cells, thereby increasing the proportion of Th17 cells in EAE mice (13). On the other hand, a high-sugar diet stimulated Th17 cell differentiation and exacerbated EAE by altering the colony structure of the gut microbiome (57).

Effects of dietary sugars on Psoriasis

Psoriasis is a chronic inflammatory skin disease characterized by abnormal proliferation and differentiation of epidermal keratinocytes (72, 73). Previous studies have shown that inflammatory adipocytokines such as IL-6 and TNF- α formed in visceral adipose tissue are key cytokines in the pathogenesis of psoriasis, so it is believed that psoriasis is related to obesity (57, 74, 75). However, new research data suggests that dietary components (simple sugars and fats), rather than obesity itself, exacerbate psoriasis (76). The researchers found that the western diet activated the interleukin 23(IL-23) signaling pathway compared with the normal diet before the mice gained weight, further increasing the production of IL-17A in $\gamma\delta$ T cells after IL-23 stimulation (76). The cytokine IL-17A is necessary for the comprehensive development of skin inflammation (77). Meanwhile, IL-23 overexpression resulted in decreased microbial diversity and pronounced dysbiosis in mice fed the Western diet (78). Even more surprising, when the mice were switched from a western diet to a standard one after IL-23 was released, skin inflammation was reduced and the gut microbiota partially reversed (78). Therefore, based on the available data, we

believe that the dysbiosis of the gut microbiota induced by short-term Western dietary intake contributes to the enhancement of psoriasis, and healthy eating pattern with less sugar should be considered for patients with psoriatic skin disease (79).

Effects of dietary sugars on inflammatory bowel disease

Inflammatory bowel disease (IBD) is a chronic inflammatory gastrointestinal disease that mainly includes two subtypes, Crohn's disease and ulcerative colitis (80). It occurs due to the interaction of multiple factors such as genetics, microbes, immune factors, modern lifestyle, and diet (81, 82). Existing research suggests that IBD affects disease severity by affecting changes in the microbial composition of the gut microbiota, while colitis microbiota shifts and alters colitis susceptibility in recipients (83). The commensal gut flora and mucus layer in the gut are known to be critical for homeostasis, as it prevents the invasion and adhesion of pathogenic microorganisms and helps maintain the integrity of the gut barrier (84). According to statistics, the incidence of IBD in Western countries is increasing, especially among children in the same period (85), indicating that the occurrence of IBD is related to Western diet and lifestyle. In recent years, IBD has also become a global health problem due to the simultaneous rise of Western diets (ie, diets high in fat and refined sugar) around the world. Recent clinical and experimental studies suggest that a high-fat diet may be a trigger for IBD, but the role of high sugar in the pathogenesis of IBD remains controversial. A landmark study shows that type 2 diabetes can lead to intestinal barrier dysfunction through transcriptional reprogramming of intestinal epithelial cells and altered tight adhesion junction integrity; it can also increase disease by causing changes in gut microbial metabolism susceptibility (86). Additionally, population-based studies have shown that about 10% of people with IBD believe that eating sugary foods trigger flare-ups and make their symptoms worse (87). In some prospective studies, consumption of HFCS and SSB have also been found to be positively associated with the risk of IBD (88–90). Taken together, the researchers believe that sugar is closely related to the composition of the gut microbiome and the occurrence and development of IBD.

Mechanisms by which dietary sugars affects inflammation

The high glucose environment is inextricably linked with the immune system, which plays an important role in immune signal and immune cell function (91). Previous study has found that high levels of glucose may lead to impaired immune system function and pathological conditions. Innate immune

macrophages, dendritic cells, and specific immune cells T cells and B cells migrate to the site of infection to protect the immune system (92). T cells are the key to cell-mediated immunity. Conventional T cells, also called $\alpha\beta$ T cells, can be differentiated into effector $CD8^+$ cytotoxic subsets and $CD4^+$ helper T cell subsets, including Th1, Th2, Th17, Tr1, Tfh, Th9, and immunosuppressive Treg cells, respectively, under the stimulation of antigen (91, 93, 94). Thaïs and his colleagues used lymphocyte culture and analysis of its $CD4^+$ and $CD8^+$ subpopulations to confirm that high concentrations of fructose can reduce lymphocyte subcomponents, resulting in a decrease in the total number of lymphocytes (95). In addition, hypertonic glucose in the peritoneal dialysis (PD) range has been reported to induce interleukin-17 (IL-17) polarization in a mitochondrial reactive oxygen species (mtROS)-dependent manner (96). Subsequently, Zhang et al. demonstrated that high glucose can activate TGF- β through ROS, and subsequently promote Th17 cell differentiation with the participation of IL-6, thereby aggravating autoimmune disease, in T cell metastasis and experimental autoimmune encephalomyelitis (EAE) induced colitis mouse models (13, 62). Therefore, high amounts of dietary sugars can lead to T cell-mediated inflammation (Figure 2). Recent studies have found that dietary components also have regulatory effects on B cells, but it is not clear which nutrients affect B cells. In order to solve this problem, Tan and his colleagues used statistical modeling to study the effects of carbohydrates, fats, and proteins on B cells, and found that carbohydrates have a great regulatory effect on B cell proliferation (97). In addition, they showed that it is glucose, but not fructose, that supports B lymphocyte generation and development, while protecting B lymphocytes from early apoptosis through activation of the mammalian target of

rapamycin signaling pathway (mTOR) (97). Additionally, a recent study mentioned the effects of a high-fructose diet (HF), high-fat diet (HFD), or both (HFHF) on leptin and ROS, but it appears that only HFHF-fed mice developed hyperglycemic symptoms, oxidative stress, and steatosis (inflammation and fibrosis), whereas HF caused only transient increases in leptin and C-peptide (98).

The gut microbiome has also been the focus of research into the effects of dietary sugars on inflammation. It mainly includes two aspects: (1) High consumption of sugars reduces microbial diversity and leads to depletion of luminal short-chain fatty acids (SCFAs) (99). SCFAs can affect the recruitment of colonic regulatory T cells and the antibacterial activity of macrophages, thereby affecting the intestinal mucosal immune system (100). The damaged intestinal barrier is unable to prevent the invasion of pathogenic microorganisms, enabling the transport of *E. coli*-derived (LPS), etc., which are recognized by their specific receptors, such as TOLL-like receptor 4 (TLR4), and activate downstream NF- κ B signaling pathway induces increased levels of inflammatory factors IL-6, IL-1 β and TNF- α and more neutrophil infiltration, leading to more severe colitis (99). (2) Shahanshah and colleagues suggest that high glucose can increase the levels of inflammatory cytokines IL-6, TNF- α , Lcn2 (Lcn2), and Cox2 (Ptgs2) by altering gut microbiota composition, mucosal association, and functional activity, thereby aggravating the progression of inflammatory bowel disease (1). In this study, Shahanshah found that mucolytic bacteria, such as *Bacillus fragilis* and *Prevotella*, were abundant in mice fed a high-glucose diet, while the relative abundance of the sugar-soluble bacteria Sutterellaceae, capable of transplanting to the epithelial barrier and inducing an inflammatory response was increased. In contrast, the

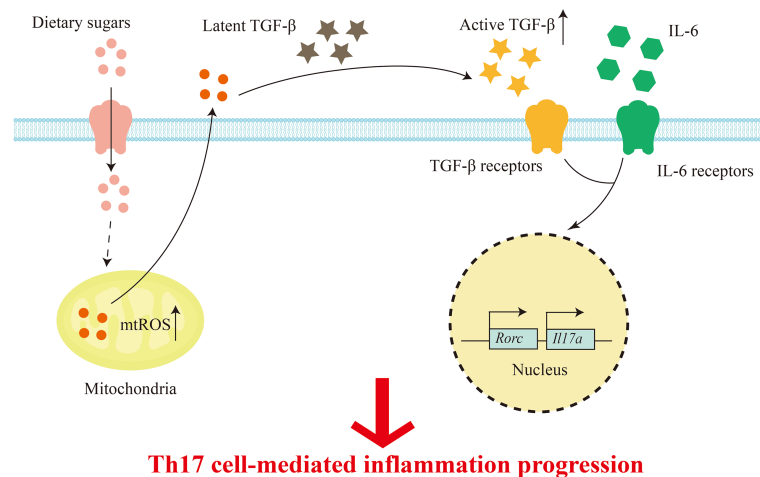


FIGURE 2

Dietary sugars-mediated T cell inflammation. Excess dietary sugars may activate TGF β through mtROS post entering T cells, and together with IL-6 in the immune microenvironment, the expression of transcription factor ROR γ t is induced to promote Th17 cell differentiation.

abundance of Lachnospiraceae and Lactobacillaceae belonging to Firmicutes decreased. Lachnospiraceae have been shown to suppress inflammation, while Lactobacillaceae are able to maintain intestinal homeostasis by inducing anti-inflammatory cytokines and protecting the intestinal epithelium from pathogens. Similarly, dietary fructose can induce intestinal inflammation by increasing intestinal cell permeability and promoting the growth of intestinal bacteria (101)(Figure 3). In addition, the effect of high fructose on the severity of IBD was abolished when gut bacteria were substantially reduced, suggesting that the changes in gut microbial composition and IBD effects of high glucose are transferable (102).

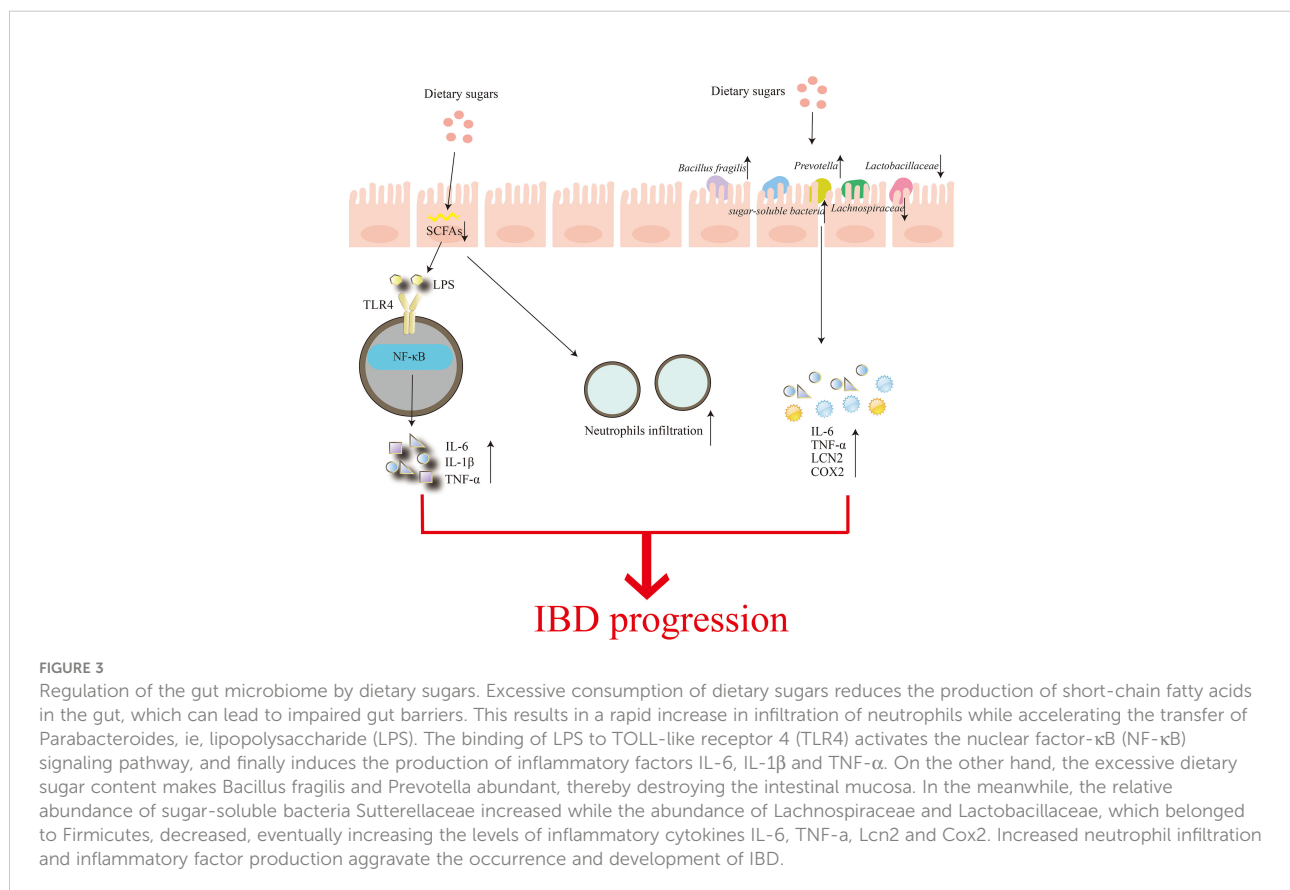
Macrophages are one of the most specialized antigens presenting cells, whose main functions are to secrete cytokines, phagocytose, and present antigens to T cells (92). The researchers found that high glucose levels induce increased expression and activity of Toll-like receptors (TLRs), which then activate NF- κ B and MAPK signaling pathways through ROS/RNS and superoxide production, leading to macrophage activation and release of inflammatory factors (Figure 4) (103, 104). High doses of glucose can induce superoxide anion production in macrophages or monocytes and promote the release of monocyte inflammatory cytokines, which up-regulate innate immune system receptors (such as TLRs) by activating NF- κ B (105). Complementary to this, high glucose

conditions impair neutrophil mobilization which is due to elevated TLRs expression (106).

Discussion

The leading cause of death in patients with diabetes is related to its accompanying complications, such as diabetic retinopathy, obesity, and cardiovascular disease (107). Inflammation and immune abnormalities are triggers for T1D and T2D and its associated complications (108, 109). When the body is attacked by an antigen, innate immune macrophages and specific immune lymphocytes are triggered to migrate to the site of infection to function, however, large amounts of glucose may lead to impaired immune system function (110). Therefore, high glucose induces a series of complications by suppressing the effective adaptive immune response generated by macrophages and T cells.

Studies have shown that dietary monosaccharide consumption is associated with T2D and cardiovascular disease, and obesity increases the risk of these diseases (110). Meanwhile, low-grade chronic inflammation is also strongly associated with obesity (39). Therefore, the association between dietary sugars and increased risk of chronic disease may be mediated by low-grade chronic inflammation. Another randomized controlled trial showed no difference in the effects of



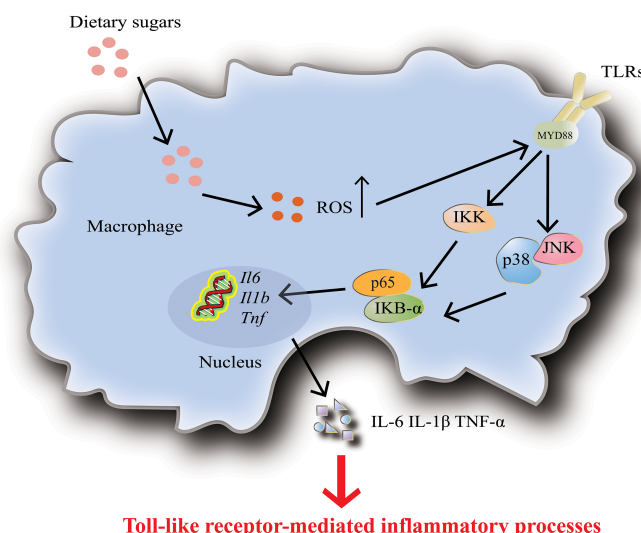


FIGURE 4

Dietary sugars-mediated inflammation in macrophages. High levels of dietary sugars lead to increased TOLL-like receptor 4 (TLR4) activity, which subsequently activates downstream the nuclear factor- κ B (NF- κ B) and MAPK signaling pathways, thereby promoting the upregulation of inflammatory factors IL-6, IL-1 β and TNF- α . In addition, dietary sugars-mediated inflammation in dendritic cells and neutrophils is also accomplished by activating TLR4.

fructose, glucose, or HFCS on obesity and systemic or adipose tissue inflammation in normal-weight adults (39). With the increasing consumption of these dietary sugars and their beverage mixes, more people around the world are suffering from systemic inflammation. A large number of studies have shown that natural small molecules widely present in plants have an inhibitory effect on systemic inflammation caused by excessive intake of dietary sugars. Studies have shown that curcumin inhibits inflammation caused by high fructose through multiple pathways. In male Wistar rat inflammatory model, curcumin can inhibit the elevation of malondialdehyde (MDA) and total oxidation state (TOS) in skeletal muscle and the expression of extracellular kinase 1/2 (ERK1/2) and P38 proteins of MAPK family members (111). Curcumin and allopurinol inhibit liver inflammation by upregulating the Mir-200A-mediated TXNIP/NLRP3 inflammasome pathway (112). Epatechin (113), astaxanthin (114), morin (115), and juglanin (116) reduce systemic inflammation by inhibiting the release of inflammatory factors IL-6, IL-1 β , and TNF- α through downstream cascades activated by TLR4 such as NF- κ B, MAPK, or JAK2/STAT3, and betulinic acid ameliorates inflammation and oxidative stress induced by high fructose diet through PIK and Akt pathways (117). In addition, S-methylcysteine (SMC) (118), spinach nitrate (119) and red ginseng mulberry leaf (MPM) (120) can inhibit inflammation induced by dietary monosaccharide overdose by inhibiting the expression of low grade chronic inflammatory markers such as serum C-reactive protein, tumor necrosis factor A, and interleukin-6 e-selectin.

Autoimmune disease is an abnormal immune response in which the immune system attacks the body's normal tissues, resulting in the chronic destruction of these tissues and severely reducing the patient's quality of life (13). Consumption of glucose-rich foods and beverages is very common in the West and may also be a key cause of the breakdown of metabolic and immune self-tolerance (62). In the new mouse model, autoimmune disease in mice can be largely alleviated if a Western diet is switched to a normal diet (121). Therefore, a reasonable and balanced dietary recommendation (low fat, low sugar) is essential for patients with autoimmune diseases. A Mediterranean diet has been proven to be more conducive to the recovery of patients with autoimmune diseases than the Western diet (63, 79). In addition to improving diet, it is hoped that dietary restrictions can improve the effects of autoimmune disease and inflammation. Recently, Dixit and his team found that insisting on a 14% reduction in long-term calorie intake can help restore thymus function, increase thymus volume, and improve the ability of the thymus to generate T cells, thereby improving immune function that typically declines with age. Phospholipase PLA2G7 may play an important role in this mechanism (122). More intriguingly, Bukhari and his colleagues found that mothers' high-fructose diets influenced neonatal immunity and altered anxiety behavior and inflammation in adolescence and adulthood (111). The study suggests that maternal diet may alter peripheral inflammation in newborns, which in turn affects anxiety-like behavior and peripheral inflammation during adolescence. These findings reveal the lasting effects of a mother's diet on her offspring's immune system, meaning that the mother's diet is crucial for their child.

Recently, it was demonstrated that excessive consumption of HFCS is associated with colon cancer development (123). In the study, mice fed with HFCS had significantly increased tumor size. This means that excess dietary sugar might be closely related to the development of tumors. However, whether immune regulation plays any key role in the tumor microenvironment remains to be explored. Overall, most of the studies were performed with mouse models, limiting the clinical applicability of these findings. Therefore, it is urgent to reveal the roles of excessive intake of hexose in the regulation of human inflammatory diseases in the future.

Author contributions

XM wrote the manuscript. FN, HL, PS, XF and XS edited the manuscript. YH and DZ supervised the work, and edited the manuscript. All authors contributed to the article and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Mechanism of inflammasomes in cancer and targeted therapies

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Inflammasomes, composed of the nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), are immune-functional protein multimers that are closely linked to the host defense mechanism. When NLRs sense pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), they assemble into inflammasomes. Inflammasomes can activate various inflammatory signaling pathways, including nuclear factor kappa B (NF- κ B) and mitogen-activated protein kinase (MAPK) signaling pathways, and produce a large number of proinflammatory cytokines, which are closely associated with multiple cancers. They can also accelerate the occurrence and development of cancer by providing suitable tumor microenvironments, promoting tumor cell proliferation, and inhibiting tumor cell apoptosis. Therefore, the exploitation of novel targeted drugs against various inflammasomes and proinflammatory cytokines is a new idea for the treatment of cancer. In recent years, more than 50 natural extracts and synthetic small molecule targeted drugs have been reported to be in the research stage or have been applied to the clinic. Herein, we will overview the mechanisms of inflammasomes in common cancers and discuss the therapeutic prospects of natural extracts and synthetic targeted agents.

KEYWORDS

inflammasomes, NOD-like receptors (NLRs), cancer, targeted therapeutics, natural extracts, synthetic small molecule targeted drugs

1 Introduction

Inflammasomes are a critical component of the innate immune system and play an essential role in defending against invasion by external xenobiotics. Unlike adaptive immunity, innate immunity is a nonspecific immunity in which immune cells have pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) from pathogenic microorganisms and damage-associated molecular patterns (DAMPs) from tissue damage to activate the immune response (1, 2). PRRs are distributed not only on the cell membrane but also in the cytoplasm. The membrane PRRs are composed of Toll-like receptors (TLRs) and C-type lectins (CTLs), while the cytoplasmic PRRs consist of the nucleotide-binding oligomerization domain (NOD)-like

receptors (NLRs), retinoic acid inducible gene-I (RIG-I)-like receptors (RLRs) and absent-in-melanoma (AIM)-like receptors (ALRs) (3–5). NLRs and ALRs can form inflammasomes, of which, NLRs dominate. Upon recognition of ligands by NLRs, it combined with the apoptosis-associated speck-like protein (ASC) and pro-caspase-1 to form the inflammasome complex. This inflammasome cleaves inactive pro-caspase-1 into active caspase-1. Caspase-1 further promotes the secretion of the pro-inflammatory cytokines IL-1 β and IL-18, which trigger inflammation by recruiting immune cells (e.g., macrophages) and inducing programmed cell death (e.g., pyroptosis) (6).

Inflammation is a defensive process of the body against injury and infection, but sustained activation of the inflammatory response may be associated with tumorigenesis and metastasis (7). The research indicated that long-term chronic inflammation is closely associated with growth inhibition resistance, angiogenesis, immune escape, malignant transformation, and metastatic potential acquisition (8). All of these processes are associated with the formation of inflammasomes. Mutations in genes encoding inflammasomes can affect the expression of nuclear factor kappa B (NF- κ B) and mitogen-activated protein kinase (MAPK) signaling pathways, which may ultimately contribute to the formation of cancer (9). The occurrence of tumors is related to the ability of cancer cells to capture inflammatory signaling pathways and promote their proliferation, migration and invasion (10). However, the exact molecular basis between inflammation and cancer remains unclear. Due to the different pathogenic mechanisms of inflammasomes in different cancers,

inflammasomes and related signaling pathways have become a current research hotspot for molecularly targeted therapies. This review highlights the research progress of NLR inflammasomes in common cancers and outlines the potential of inflammasomes and key molecules in signaling pathways as therapeutic targets.

2 Structure and classification of the NLR family

The NLR proteins consist of three distinct parts, the NACHT domain in the central region, flanked by leucine-rich repeats (LRRs) at the C-terminus, and an effector domain at the N-terminus (Figure 1). The NACHT domain has dNTPase activity and is involved in ATPase-dependent oligomerization (11, 12). LRRs can sense a variety of afferent signals. For example, NOD1 and NOD2 can detect bacterial peptidoglycan (13), NLRC4 and NAIP sense type III secretion system (T3SS) and flagellin (14), NLRP3 can be activated by sensing other molecules, such as ATP, reactive oxygen species (ROS), silica, uric acid crystals and nigericin (15), but the exact mechanisms by which it senses ligands are not yet clarified. The N-terminal effector domain is used to mediate the signal transduction of downstream targets, thereby activating caspases and various signaling pathways (16).

According to the different N-terminal effector domains, NLRs family is divided into 4 subfamilies, containing a total of 22 members (Figure 1). Among them, NLRA subfamily has only one member, the class II major histocompatibility complex

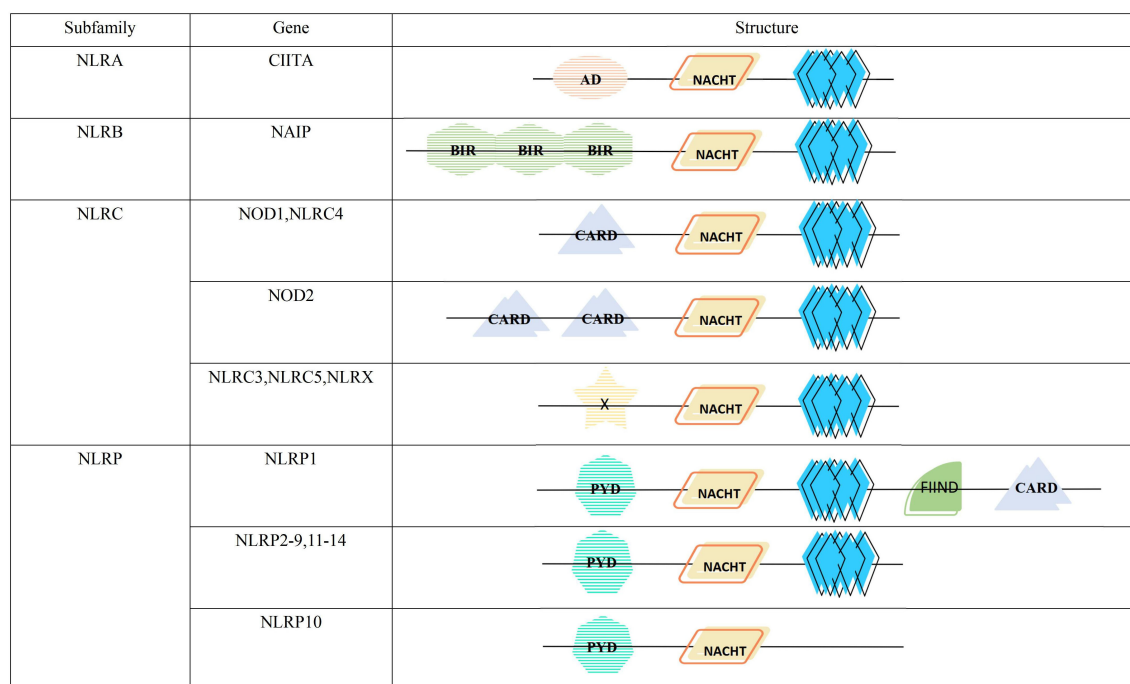


FIGURE 1

Structure and classification of the NLR family. AD, acidic transactivation domain; NACHT, NACHT domain (consisting of seven distinct conserved motifs, including the ATP/GTPase-specific P-loop, the Mg²⁺-binding site, and five more-specific motifs); BIR, baculovirus inhibitor of apoptosis repeat; CARD, caspase activation and recruitment domain; X, unidentified; PYD, pyrin domain; FIIND, function to find domain; leucine-rich repeat.

transactivator (CIITA), containing the acidic transactivation domain (AD). It can enhance the transcription of MHC class II molecules and inhibits the classical NF- κ B pathway (17). NLRB subfamily, characterized by a baculoviral inhibitory repeat-like domain (BIR), is also comprised of a single member, the NLR family apoptosis inhibitory protein (NAIP). It is responsible for preventing apoptosis mainly by inhibiting the activities of caspase-3, caspase-7 and caspase-9 (18). NOD1, NOD2 and NLRC4 with the caspase activation and recruitment domain (CARD), and NLRC3, NLRC5 and NLRX1 with unknown domains all belong to NLR subfamily. The former can monitor microbial invasion and affect cytokines secretion by upregulating or downregulating inflammatory signaling, while the latter regulates autophagy and cell death by fine-tuning the cascade response of inflammatory signaling and the type I IFN signaling pathway (19). NLRP containing pyrin domain (PYD) has 14 members, namely NLRP1-14, which are mainly responsible for the modulation of inflammatory signaling and apoptosis (20).

3 Activation pathways of inflammasomes

Inflammasomes are a set of multi-protein complexes assembled with the participation of natural immune recognition receptors, which function as an inflammatory immune response by secreting inflammatory cytokines and inducing cellular pyroptosis (21). “The canonical inflammasome pathway” was defined when the inflammasome activation was mediated by caspase-1, while “The noncanonical inflammasome pathway” was classified when the inflammasome activation was dependent on human caspase-4/5 or mouse orthologue caspase-11 (22, 23). These two activation pathways and their roles are described in detail below.

3.1 The canonical inflammasome pathway

Activation of the inflammasomes requires the involvement of “activators”. In the absence of activator stimulation such as DAMPs and PAMPs, LRRs interact with the NACHT domain and inhibit inflammasome formation (24). When LRRs detect DAMPs, PAMPs or environmental stimuli in microbes, the environment or the organism, NLR proteins start recruiting the adaptor protein ASC, which interacts through the Pyrin-Pyrin structural domain (25). Then the effector domain pro-caspase-1 binds to ASC through the CARD-CARD domain to assemble into an inflammasome, and finally, oligomerization to form an inflammasome complex possessing a heptamer (26). Since NLRP1 itself possesses a CARD domain, it can form inflammasomes directly through the interaction of the CARD-CARD domain with pro-caspase-1 without the need for ASC (27). Nonetheless, related studies have shown that ASC can enhance the stability of the CARD-CARD domain between NLRP1 and pro-caspase-1, thereby driving the immune response (28). Thus, ASC is an essential component of the inflammasomes complex assembly process, and when the body is

deficient in ASC, it can lead to a variety of diseases (29, 30). NLRC4 does not contain a Pyrin structural domain, and it functions primarily by interacting with NAIP to form NAIP-NLRC4 inflammasomes. First, ligands such as flagellin or bacterial type III secretion system (T3SS) interact with non-activated NAIP, causing a conformational change and thus activation. Then ligands with activated NAIP further cause a conformational change in non-activated NLRC4, and activated NLRC4 can recruit more NLRC4 monomers to form NAIP-NLRC4 inflammasomes (31). Inflammasome assortment can further mediate the formation of active caspase-1 by self-hydrolysis of inactive pro-caspase-1, which can cleave pro-IL-1 β and pro-IL-18 to form the mature inflammatory cytokines IL-1 β and IL-18 (32). Inflammatory cytokines recruit immune cells, such as T lymphocytes and neutrophils, at the sites of infection and inflammation, thereby regulating the innate and adaptive immune response. They also initiate autocrine and paracrine inflammatory signaling cascades, providing survival signals to normal cells to prolong their lifespan and death signals to abnormal cells to accelerate their death (33). In addition, activated caspase-1 cleaves gasdermin D (GSDMD) and releases its N-terminal domain, which translocates to the cell membrane to form a pore that mediates the release of cellular contents such as IL-1 β and IL-18, and induces pyroptosis (34) (Figure 2).

3.2 The noncanonical inflammasome pathway

The noncanonical inflammasome pathway is triggered by caspase-4/5/11. When TLR4 of the organism senses the lipopolysaccharide (LPS) of Gram-negative bacilli, it promotes the transcription of pro-IL-1 β and pro-IL-18 by inducing the activation of NF- κ B (35). In addition, when signaling to Trif/IRF3 induces the expression of type-I-IFNs, which promotes the secretion of pro-caspase-11. But it remains unknown whether the activation of caspase-11 is due to other additional signals or because pro-caspase-11 is automatically activated after reaching a certain threshold (36). Activated caspase-11 activates the NLRP3 inflammasome to induce caspase-1-dependent maturation and secretion of IL-1 β and IL-18, but the activation pathway remains to be determined. In addition, caspase-11 can also cleave GSDMD to induce caspase-1-independent pyroptosis (21). Excessive pyroptosis can lead to a variety of cancer-related diseases, such as gastric cancer, colorectal cancer, and ovarian cancer (37) (Figure 2).

4 Role of NLR inflammasomes in tumor development and metastasis

Many studies have demonstrated that abnormal activation of inflammasomes is closely associated with several human diseases, for instance, gout, silicosis, periodic fever syndrome, Crohn's disease, type 1 diabetes, and so on (6, 38, 39). Currently, the increasing evidence shows that the inflammatory response

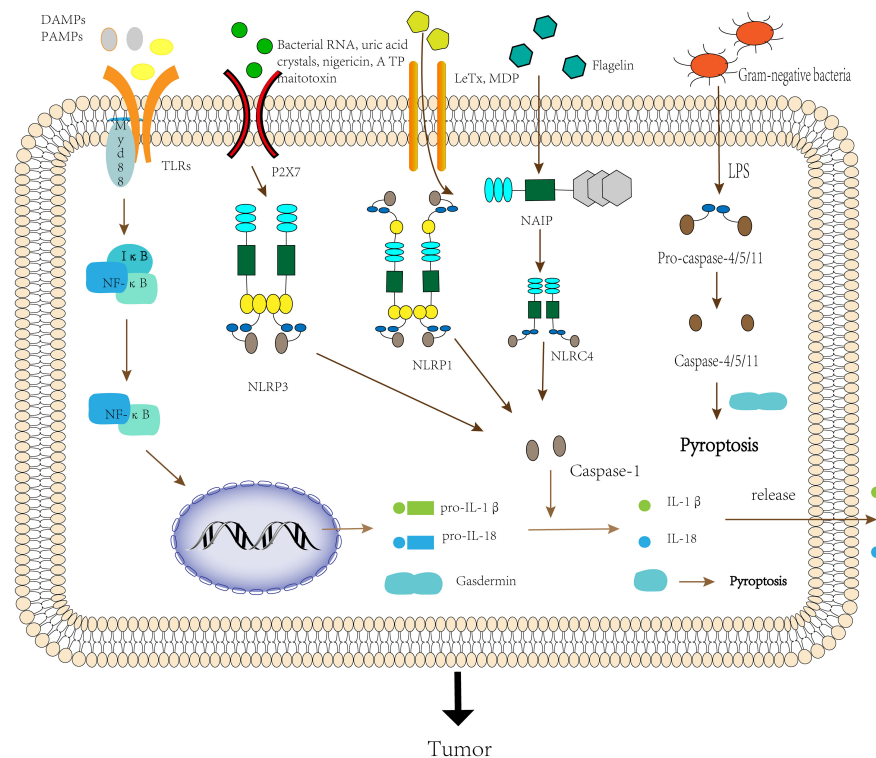


FIGURE 2
The activation process of the inflammasome.

mediated by NLR inflammasomes is involved in regulating physiological processes such as tumor development and metastasis. When the inflammasomes are abnormally activated, a variety of inflammatory cytokines and chemokines, including TNF- α , IL-1 β , IL-6, IL-18, CCL2/MCP-1, are excessively secreted, which affect the development of cancer (40, 41). Among them, TNF- α plays a pro-tumor role by promoting DNA damage and inhibiting DNA repair (42). The expression of IL-1 β and IL-18 is significantly elevated in a variety of malignancies, and these cytokines can promote cancer development and distant metastasis by triggering the secretion of VEGF, FGF2 and STAT3 (43, 44). IL-6 can promote the invasive ability of cancer cells, which leads to tumor metastasis. But there are some studies with contradictory findings, probably because inflammasomes not only promote tumor progression, but also correlate with apoptosis of tumor cells (45). The following will review the effects and mechanisms of inflammatory response mediated by NLR inflammasomes on common tumors including colorectal cancer, breast cancer, liver cancer and melanoma.

4.1 NLR inflammasomes and colorectal cancer

Colorectal cancer (CRC) is a malignant tumor occurring in intestinal epithelial cells, and the most common pathological carcinoma is adenocarcinoma. The overall incidence rate ranks in the top three among malignant tumors, and the mortality rate ranks the fourth, after lung, liver, and stomach cancers (46, 47). Reducing

the prevalence and mortality of CRC has become an urgent issue to be solved. At present, surgical treatment, neoadjuvant chemoradiotherapy and adjuvant chemotherapy are the main clinical treatment methods. With the development of endoscopic technology and various auxiliary technologies, the 5-year survival rate of early-stage CRC can be as high as 92% (48, 49). However, there is still no effective treatment to reduce the prevalence. The search for new treatment methods and new therapeutic targets is currently a hot topic in academic circles.

Colorectal cancer model is the most studied cancer model, and a number of studies have shown that a variety of NLR inflammasomes are closely related to it, among which NLRP3, NLRP4, NOD2, NLRP1, NLRP6, NLRP12 inflammasomes have protective effects on colorectal cancer, while the effects of NLRP3 and NOD1 inflammasomes are still controversial (50). Rajendra's team (51) found that NLRP3 inflammasomes modulated the multiplication and differentiation of stem cells and could promote apoptosis. What's more, the upregulation of *Nlrp3* was negatively correlated with the occurrence of tumors. In contrast, in *Nlrp3* downregulated enterocytes, cell proliferation was not controlled due to the inability to inhibit the activation of the P13K-mTOR signaling axis, which may lead to tumorigenesis (52). Similarly, in a mouse model of colorectal cancer, the expression of *Nlrp4* mRNA was diminished (50). Compared with wild-type mice, *Nlrp4*^{-/-} mice could promote the proliferation of colonic epithelial cells and inhibit apoptosis of tumor cells, thereby promoting tumor formation, suggesting a protective effect of NLRP4 inflammasomes on colorectal carcinogenesis (53). In addition,

Allam's team (54) concluded that the higher incidence of colorectal cancer in *Naip*^{-/-} mice may be related to the inability of *Naip*^{-/-} mice to suppress the overactivation of STAT3, a transcription factor that promotes tumor growth. STAT3 hyperactivation was not observed in *Nlrp4*^{-/-} mice, demonstrating that the protective effect of NAIP inflammasomes may be independent of NLRC4 inflammasomes. Couturier et al. (55) found an increased distal colon tumor load in *Nod2*^{-/-} mice by comparison with wild-type mice. This phenomenon may be due to an imbalance of pro- and anti-inflammatory cytokines and a loss of autophagy and apoptosis, leading to chronic inflammation as well as an increased risk of cancer (56). Nashir (57) and his team concluded that NOD2 inflammasomes could inhibit CRC by blocking IRF4, thereby downregulating NF- κ B and MAPK signaling pathways. NLRP1 and NLRP6 inflammasomes prevent CRC by mediating the production of IL-1 β and IL-18 through caspase-1 (58–60). In addition, it has been shown that CCL5-induced inflammation can increase the number of tumors in *Nlrp6*^{-/-} mice (61). The team of Zaki (62) found that NF- κ B, ERK, and STAT3 signaling pathways were highly activated and that tumor incidence was increased in the colon of *Nlrp12*^{-/-} mice. The ERK pathway activates the oncogenic transcription factor cMyc, which activates multiple oncogenic factors including COX. STAT3 regulates the pro-inflammatory cytokines IL-17 and IL-23, the anti-apoptotic protein Bcl-xL, and a variety of growth factors. Allen (11) also found that classical and non-classical NF- κ B signaling pathways were activated and the body was highly susceptible to colorectal cancer in *Nlrp12*^{-/-} mouse models. Among them, NLRP12 interacted with NIK in the non-classical NF- κ B pathway and inhibited the production of p52, making this pathway dysregulated. Compared to the non-classical NF- κ B pathway, the classical pathway has a relatively weak effect. In addition, NLRP12 inflammasomes also was able to regulate MAPK and AKT pathways, resulting in reduced tumorigenesis in mouse models (62). Stefanie's group (63) found an interesting phenomenon in that NLRP5 inflammasomes expression was not detected in normal intestinal tissues, but it expressed in tissues with intestinal cancer, which indicated that NLRP5 inflammasomes expression was associated with tumorigenesis. However, the specific mechanism of action between NLRP5 inflammasomes and CRC needs to be further explored. The occurrence of CRC is probably related to immunological factors such as the activation of immune response and recruitment of immune cells. NLRP3 was found to be expressed in both immune cells and epithelial cells of colon cancer, manifesting that NLRP3 inflammasomes are involved in the formation of CRC (64). However, this aspect of research is still controversial: some studies have shown a positive correlation between NLRP3 inflammasomes and CRC, but a part of the research holds the opposite view, they suggest that NLRP3 inflammasomes may inhibit tumor development (65). Zaki et al. (66) found that NLRP3 inflammasomes lacking the adaptor proteins ASC and caspase-1 played a protective role in CRC compared to wild-type mice. Another study demonstrated that the use of NLRP3 inflammasomes small molecule inhibitors reduced the incidence of CRC (67). In addition, it has been reported that NOD1 inflammasomes deficiency caused damage to the intestinal epithelial barrier, increased apoptosis, and release of

inflammatory cytokines in mouse models, leading to the development and progression of CRC (68, 69). Conversely, some other authors have argued that NOD1 is highly expressed in CRC mice and patients, and increased NOD1 expression would decrease the long-term survival of patients. NOD1 inflammasomes enhances tumor cell adhesion, migration and metastasis by activating MAPK signaling pathway (70). Tumorigenesis and metastasis require an appropriate microenvironment, and Charles et al. (71) suggested that NOD1 expression on myeloid cells formed a microenvironment conducive to tumor growth and may contribute to the development of CRC (Table 1).

4.2 NLR inflammasomes and breast cancer

Among female malignancies, breast cancer ranks first and has become one of the major public health problems in the world (89, 90). With the increasing improvement of breast cancer treatment methods, in addition to traditional surgery, adjuvant chemoradiotherapy and hormone therapy, targeted gene therapy and immunotherapy have also been used in clinical practice. Although the 5-year survival rate of patients has improved significantly, the mortality rate is still high (91, 92). It is of great clinical importance to find new therapeutic targets and develop new preventive and therapeutic measures to reduce the morbidity and mortality of breast cancer.

There have been many studies showing that abnormal expression of multiple NLR inflammasomes are closely connected with breast cancer. The NLRC4/IL-1 β signaling pathway has been shown to promote the progression of breast cancer. In adipocytes, NLRC4 inflammasomes induce tumor infiltration into myeloid cells, and IL-1 β promotes vascular endothelial growth factor A (VEGFA) secretion and angiogenesis, thereby driving disease progression (72). A study showed that NAIP was expressed in breast cancer and at significantly higher levels than in tumor control groups. In addition, NAIP was overexpressed in patients with poor outcomes and poor prognoses. These results suggest an aggravating role for NAIP inflammasomes in breast cancer (73). Nour (75) et al. proposed that the NLRP3/IL-1 β pathway was strongly connected with the development and metastasis of breast cancer. Compared with normal breast tissue, the expression of *Nlrp3*, *caspase-1* and *IL-1 β* genes in the NLRP3 inflammatory pathway was significantly upregulated in mouse and human mammary tumor stroma. Among them, NLRP3 inflammasomes could mediate infiltration of CD11b + Gr1+ immune cells into mammary tumor tissues, and IL-1 β could promote mammary tumor progression and tumor cell metastasis to the lung by upregulating adhesion molecules expression in primary tumors and metastatic sites. Guo et al. (76) also made similar findings that IL-1 β secretion and tumor cell metastasis to the lung were reduced in *caspase-1*^{-/-} and *Nlrp3*^{-/-} mice compared to wild-type mice. What's more, blocking IL-1R with inhibitors inhibited tumor growth and metastasis. The authors posited that this may be because activation of inflammasomes and production of IL-1 β could recruit myeloid-derived suppressor cells (MDSC) and tumor-associated macrophages to tumor tissues, creating a favorable microenvironment for tumor metastasis. However,

François et al. (74) held the opposite view, arguing that NLRP3 inflammasomes exerted anti-tumor functions by stimulating IL-1 β release from dendritic cells. It has been proposed that NLRP1 inflammasomes promotes proliferation, migration and invasion of the breast cancer cell line MCF-7 compared to normal breast tissue. IL-1 β , IL-18 and ASC, the pivotal elements of the inflammatory signaling pathway, are upregulated in breast cancer cells (77), but the specific cancer-promoting mechanism of NLRP1 inflammasomes needs to be further verified. Some investigators suggested that NOD1 and NOD2 inflammasomes may also be relevant to the negative regulation of breast cancer. In a study of estrogen receptor(ER)-negative Hs578T cell line, the investigators found that the proliferation rate of cells overexpressing NOD1 was reduced *in vitro*, and a similar phenomenon was seen in NOD2 overexpressed cells, even more significant than the former, which may be the result of activation of MAPK pathway signaling (93). Another *in vitro* cellular experiment showed that in the ER-positive MCF7 breast cancer cell line, NOD1 upregulation promoted RIP2 and caspase-8 mediated apoptosis and decreased estrogen-induced cell proliferation response (94). In addition, NOD1 downregulation

enhanced the multiplication of breast cancer cells (80). Therefore, NOD1 inflammasomes was considered to be a tumor suppressor in ER-positive breast cancer cells (Table 1).

4.3 NLR inflammasomes and liver cancer

Liver cancer is the fifth most common cancer, with a mortality rate of 9.1% of all cancers. It is estimated that there were about 780,000 new cases of liver cancer and nearly 740,000 deaths in 2012 (78). Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) are the two main histological types of liver cancer, of which 70%-85% are HCC and 10% - 20% are ICC (79, 95). There are many causes of hepatocellular carcinomas, such as immune activation due to chronic inflammation, bacterial translocation of intestinal flora and damage to hepatocytes by endotoxins secreted by them, etc. (96, 97). Most researchers now believe that certain genetic mutations, upregulation or downregulation of gene expression due to chronic inflammation caused by hepatitis viruses, such as hepatitis B virus (HBV) or

TABLE 1 Effects of NLR inflammasomes in cancer.

Cancer	NLR inflammasomes	Effect	References
Colorectal cancer	NLRC3	Protection	(51, 52)
	NLRC4	Protection	(53)
	NOD2	Protection	(56, 57)
	NLRP1	Protection	(60)
	NLRP6	Protection	(58, 61)
	NLRP12	Protection	(62)
	NLRP3	Protection	(65, 66)
	NLRP3	Aggravation	(64)
	NOD1	Protection	(68, 69)
	NOD1	Aggravation	(70, 71)
Breast cancer	NLRC4	Aggravation	(72)
	NAIP	Aggravation	(73)
	NLRP3	Protection	(74)
	NLRP3	Aggravation	(75, 76)
	NLRP1	Aggravation	(77)
	NOD1	Protection	(78, 79)
	NOD2	Protection	(80)
Liver lung	NLRP3	Protection	(81)
	NLRC5	Aggravation	(82)
	NLRP12	Protection	(83, 84)
Melanoma	NLRP1	Aggravation	(85)
	NLRP3	Aggravation	(86)
	NLRC4	Protection	(87)
	NLRC4	No effects	(88)

hepatitis C virus (HCV), may further contribute to the development of cancer, which is considered a potential risk factor for hepatocellular carcinoma (98). Significant genetic variants in NLRs were identified in a genomic sequence study, among which the gene mutations of NLRP3, NLRC5, NLRP12 and activation of their signaling pathways were widely noted (99, 100) (Table 1).

Raised levels of NLRP3 inflammasomes were observed in patients with chronic HBV infection, indicating that NLRP3 inflammasomes is an important intracellular receptor for HBV infection and that it may induce inflammatory responses by stimulating the secretion of IL-1 β and IL-18 (101). In patients suffering HCV, the NLRP3 inflammatory signaling pathway has also been implicated as a factor stimulating elevated serum IL-1 β levels (100). In a clinical study, NLRP3 expression was found to be upregulated in hepatitis and cirrhosis but downregulated in HCC (102). Another study found that human stanniocalcin-1 could reduce the volume of HCC tumor tissue by upregulating NLRP3 signaling pathway (103). This implies that NLRP3 inflammasomes are negatively associated with HCC. NLRC5 inflammasomes can promote HCC, it has been shown that knocking out *Nlrp5* can suppress tumor growth and metastasis by targeting the Wnt/ β -catenin signaling pathway (104). In addition, NLRP12 inflammasomes also influence the progression of HCC. A proto-oncogene cJun exists in hepatocytes, and JNK, a cJun N-terminal kinase, is prominently activated in hepatocellular carcinoma. NLRP12 inflammasomes can inhibit HCC by suppressing the JNK signaling pathway. Additionally, they can mediate NF- κ B downregulation and ERK activation, which has negative regulatory effects on HCC (81). Hepatocyte-specific cytokines include chemokines such as CXCL1, CXCL2 and CCL2, which enhance inflammatory cell infiltration, thereby increasing inflammation in the tumor environment. It has been found that NLRP12 inflammasomes can induce the downregulation of hepatocyte-specific cytokines and exert an inhibitory effect on HCC (82) (Table 1).

4.4 NLR inflammasomes and melanoma

Melanoma is one of the fastest growing cancers in the world and the most aggressive of all skin cancers, with an increasing incidence worldwide in recent years (83, 84). It is a malignant tumor derived from the malignant transformation of melanocytes and usually occurs in the skin and mucous membranes. Surgery is the radical treatment for most early melanoma, and the treatment of metastatic melanoma includes molecularly targeted therapy and immunosuppressive therapy. However, when it comes to patients who do not respond to immunotherapy, who do not have appropriately targeted drugs, who have relapsed, or who have failed to exhaust their available treatment options, their mortality rate is very high (105). Therefore, finding new therapeutic targets is of great importance to prolong the long-term survival of patients.

NLRP3 and NLRP1 inflammasomes polymorphisms were associated with susceptibility to melanoma in a Swedish case-control study (106). Zhai (107) mentioned in his paper that NLRP1 expression was upregulated in melanoma cells. Moreover,

in *Nlrp1*^{-/-} metastatic melanoma cells, decreased inflammatory cytokines secretion and NF- κ B activity were observed, while increased caspase-2/-9 activity and promoted apoptosis. These phenomena indicated that NLRP1 inflammasomes promote melanoma growth. Similarly, NLRP3 inflammasomes promote melanoma growth by activating caspase-1 and producing IL-1 β , which leads to suppression of anti-tumor immunity generated by NK cells and T cells (9, 108). The role of NLRC4 inflammasomes in melanoma is controversial. A study showed that subcutaneous injection of B16F10 melanoma in *Nlr4*^{-/-} mice accelerated tumor growth (85). The authors also observed that the production of inflammatory factors, chemokines and IFN- γ was reduced in the *Nlr4*^{-/-} model, which could be responsible for the accelerated growth of the tumors. Interestingly, another study used the same mouse model, but there was no discrepancy in neoplasm incidence between wild-type and *Nlr4*^{-/-} mice from the same litter (86). This may be due to the fact that littermate mice largely eliminate the effects of confounding factors such as gut microbiota and genetic differences (53) (Table 1).

5 Targeted therapies targeting the NLR inflammasomes

The excessive activation of the inflammasome has been shown to be directly related to a variety of autoimmune diseases and cancers. Therefore, the development of targeted therapeutic drugs targeting the inflammasomes and key molecules in their signaling pathways is very promising. More than 50 therapeutic agents targeting inflammasomes and key molecules in their signaling pathways are reported to be still in development or already on the market (87). Most of them are inhibitors of NLRP3 and IL-1. Currently, there are two main classes of drugs targeting inflammasomes, namely natural extracts and synthetic small molecule agents.

5.1 Natural extracts

Sulforaphane, a compound extracted from cruciferous vegetables, exerts biological effects by inhibiting the activation of NLRC4 and NLRP3 inflammasomes and the secretion of IL-1 β (88). Due to its anti-inflammatory and anti-tumor properties, it is currently in clinical trials for the treatment of prostate cancer (109) and breast cancer (88). It has been shown that in 20 patients of recurrent prostate cancer treated with sulforaphane, the time to Prostate-specific antigen (PSA) doubling was longer than that before treatment (6.1 months before treatment vs. 9.6 months during treatment ($p = 0.044$)) (109), indicating that the progression of the disease was slowed down. Andrographolide is a natural diterpene compound isolated from *Andrographis paniculata*. It inhibits the activation of inflammasomes mediated by NF- κ B, TNF- α and mitochondrial autophagy, resulting in reduced secretion of IL-1 β and IL-6, thereby delaying the progression of colon cancer and reducing the tumor load (67,

110). GL-V9 is a derivative of baicalein, which degrades NLRP3 inflammasomes by inducing autophagy, and can also delay colon cancer progression and tumorigenesis (111). Fumigaclavine C is a natural toxin derived from the marine-derived *Aspergillus fumigatus*. Guo (112) et al. suggested that it could reduce the occurrence of colitis and delay the progression of colon cancer in mice by inhibiting the NLRP3 signaling pathway and downregulating TNF- α , IL-1 β and IL-17A. Li et al. (113) mentioned that Fumigaclavine C could significantly downregulate the expression of NF- κ B, inhibit the MAPK signaling pathway and induce caspases-3, -8 and -9-mediated apoptosis to inhibit breast cancer development, thus it may be used as a targeted therapy for breast cancer. In addition, Caffeic acid phenethyl ester, a natural component isolated from propolis, has been proposed as a potential therapeutic component. It further inhibits caspase-1 activation and IL-1 β production by inhibiting NLRP3, thereby suppressing the development of inflammation (114). Besides the above drugs, many botanical drugs also target NLRP3 inflammasomes, such as Withaferin A (115) and Mangiferin (116). Beyond that, there are a lot of plant extracts that target other NLRs inflammasomes, such as apigenin, a flavonoid, that protects against colitis in mice by inhibiting the NLRP6 signaling pathway, which is currently in clinical trials for CRC and breast cancer (117) (Table 2).

5.2 Synthetic small molecule agents

In recent years, several small molecules targeted agents have been identified that act directly on inflammasomes with the advantages of greater specificity, lower cost and less invasiveness, and have been of increasing interest to researchers. Here, several NLRP3 inflammasomes inhibitors are introduced. MCC950, was found to specifically inhibit classical and non-classical NLRP3 inflammasome activation pathways in macrophages *in vitro* (118). However, it has been found to have serious liver toxicity in phase II clinical trials, so it is not currently used in clinical practice (119). OLT1177, another NLRP3 inflammasome inhibitor based on MCC950, not only avoids the occurrence of liver injury mentioned above, but also can treat inflammation-related diseases by inhibiting NLRP3 inflammasome activation (120). It has been shown that patients with Cryopyrin-associated periodic syndromes (CAPS) treated with high concentrations of OLT1177 for 8 consecutive days have no liver injury and a significant reduction in blood inflammatory factors (120). OLT1177 not only plays a role in the treatment of inflammation-related diseases, but also has a promising future in the field of tumor. Inhibition of the NLRP3 inflammasomes blocked the process of caspase-1 activation, ultimately leading to reduced production of the inflammatory factors IL-1 β , IL-6. This process has been shown to be related to the reduction of tumor cell proliferation (121). Oridonin is a small molecule derived from *Rabdosia rubescens*, and current studies have revealed that it can suppress the interaction between NLRP3 and NEK7, thereby inhibiting NLRP3 inflammasomes activation and oligomerization, but it does not affect AIM2 and NLRC4 (122). It has been shown that Oridonin can limit the proliferation of cancer cells in breast and ovarian cancers. Besides, Bay 11-7082 and

Tranilast are also currently developed specific NLRP3 inflammasomes targeting inhibitors (123, 124) (Table 2).

Currently, three drugs, canakinumab, anakinra and rilonacept, are approved by the US Food and Drug Administration as targeted therapy for IL-1 (125). Among them, canakinumab is an IL-1 β antibody, which has been reported to significantly reduce lethality in lung cancer patients (126). In addition, clinical efforts are also underway to study the efficacy of canakinumab in other cancers, such as non-small cell lung cancer (NSCLC) and triple-negative breast cancer (TNBC) (127). Anakinra is a recombinant IL-1 receptor antagonist that exerts biological effects by directly blocking the binding of IL-1R and IL-1. At present, it has been used to treat a variety of diseases such as myeloma and mCRC (128, 129). Furthermore, various clinical trials have proven that its use in combination with other chemotherapeutic agents can significantly improve patient prognosis and increase survival rates (130). Moreover, rilonacept is a blocking receptor that binds both IL-1 β and IL-1 α , which is currently approved for the treatment of CAPS (131). Given the preliminary therapeutic effects of these approved biologics on NLRP3-related inflammatory diseases, clinical studies and drug development of the aforementioned IL-1 blockers are steadily progressing (Table 2).

P2X7 receptor plays an important role in the NLRP3/caspase-1 cascade, which can promote tumor proliferation, migration and invasion (132). Bevacizumab, a P2X7 receptor antagonist, has an inhibitory effect on tumor growth (133). As early as 2004, it has been approved by the United States for the treatment of metastatic CRC(mCRC). At present, it has been approved for a variety of solid tumors, such as non-small cell lung cancer, renal cell carcinoma, and glioblastoma. Studies have shown that compared with chemotherapy agents (irinotecan, fluorouracil and leucovorin) alone, Patients with mCRC treated with bevacizumab combined with chemotherapy had significantly longer survival time (6.2 months vs10.6 months; $p < 0.001$) (134). Another study showed that the combination of bevacizumab and carboplatin plus paclitaxel in patients with non-small cell lung cancer reduced the risk of death by 21%, as compared with chemotherapy alone (135). What's more, AZD9056 and Glyburide are also the P2X7 receptor antagonist. The former is used to treat autoimmune diseases such as rheumatoid arthritis and osteoarthritis, and the latter is used to treat type 2 diabetes (136) (Table 2).

6 Future and prospects

This review focuses on the biological effect of inflammasomes and the targeted therapeutic agents that aim at inflammatory signaling pathways in various cancers. In recent years, we have gained a deeper insight into the role of NLR inflammasomes, which go far beyond the simple recognition of and fight against pathogens outside the organism, and have demonstrated that NLR inflammasomes-mediated inflammatory signaling pathways are closely associated with all stages of cancer development and metastasis. Sustained activation of inflammasomes can promote the secretion of inflammatory cytokines, leading to increased immune cell infiltration, thereby changing the tumor

TABLE 2 The agents targeting inflammasomes for cancer treatment.

Compounds	Mechanisms of action	Studies in cancer	Status
1. Natural extracts			
Sulforaphane	Inhibit the activation of NLRC4 and NLRP3 inflammasomes and the secretion of IL-1 β .	Reduce the PSA level of patients with recurrent prostate cancer. Inhibit the proliferation of breast cancer cells in breast cancer patients.	Phase I/II
Andrographolide	Inhibit the activation of NLRP3 inflammasomes mediated by NF- κ B, TNF- α and mitochondrial autophagy; Reduce secretion of IL-1 β and IL-6.	Reduce the risk of CAC.	Phase I/II
GL-V9	Degrade NLRP3 inflammasomes by inducing autophagy.	Downregulate the CAC tumor number, size and average tumor burden in C57 BL/6 mice model.	Pre-clinical
Fumigaclavine C	Inhibit the NLRP3 signaling pathway and downregulate TNF- α , IL-1 β and IL-17A; Downregulate the expression of NF- κ B, inhibit the MAPK signaling pathway and induce caspases-3, -8 and -9-mediated apoptosis.	Delay the progression of colon cancer in mice model. Inhibit breast cancer development in mice model.	Pre-clinical
Caffeic acid phenethyl ester	Inhibit caspase-1 activation and IL-1 β production through suppressing NLRP3.	–	–
Apigenin	Inhibit the NLRP6 signaling pathway.	Clinical trials in CAC and Breast cancer patients are Ongoing.	Pre-clinical
2. Synthetic small molecule agents			
MCC950	Inhibit classical and non-classical NLRP3 inflammasome activation.	Because of its serious liver toxicity in phase II clinical trials, so it is not currently used in clinical practice.	–
OLT1177	Block the process of caspase-1 activation by inhibiting the NLRP3 inflammasome, ultimately leading to reduced production of the inflammatory factors IL-1 β , IL-6.	Reduce infiltration of myeloid-derived suppressor cells and increased CD8+ T cells and NK cells, increase efficacy of metastatic breast cancers.	Pre-clinical
Oridonin	Suppress the interaction between NLRP3 and NEK7, thereby inhibiting NLRP3 activation and oligomerization.	Limit the proliferation of cancer cells in breast and ovarian cancers.	Pre-clinical
Canakinumab	Anti-IL-1 β antibody.	Lung cancer mortality is significantly lower in the canakinumab 300 mg group than in the placebo group. And the incident lung cancer is significantly less frequent in the 150 mg and 300 mg groups.	Launched
Anakinra	Block the binding of IL-1R and IL-1.	Decrease IL-17A levels, increase IFN- γ release, increase CD8 infiltration and showed durable tumor stabilization efficacy in mCRC patients.	Launched
Rilonacept	Block the binding of IL-1 β and IL-1 α .	It is currently approved for the treatment of inflammatory diseases such as CAPS, but not yet for cancer.	Launched
Bevacizumab	Antagonize the P2X7 receptor.	It can significantly prolong the survival time of mCRC patients. Reduce the risk of death by 21% in patients with non-small cell lung cancer.	Launched
AZD9056	Antagonize the P2X7 receptor.	It is currently approved for the treatment of inflammatory diseases such as rheumatoid arthritis and osteoarthritis, but not yet for cancer.	Phase II
Glyburide	Inhibit the activation of NLRP3 inflammasomes; Antagonize the P2X7 receptor.	Because of the risk of hypoglycemia, it has not been used in the treatment of cancer and is mainly used in the treatment of type 2 diabetes.	Launched

microenvironment. In addition, inflammasomes can inhibit T-cell and NK cell-mediated antitumor activity, all of which can accelerate cancer progression. It has been found that inflammasomes also have vital roles in inhibiting tumorigenesis development, which provides a theoretical basis for the development of novel anticancer drugs.

Since the intricate relationship between inflammasomes and various types of cancer is difficult to elucidate, the mechanism of inflammasomes in cancer and whether they can be potential therapeutic targets for cancer have attracted extensive attention.

Currently, a variety of natural plants targeting key molecules in the NLR inflammasomes signaling pathway have been developed for the treatment of inflammatory diseases (115, 116, 137, 138). Nevertheless, their exact mechanisms in the activation of inflammasomes and their anti-cancer effects still need further investigation. What's more, a number of synthetic inflammasomes antagonists and monoclonal antibodies are used to modulate inflammasomes activity, thereby inhibiting tumor development and metastasis (139, 140). One of them, targeting

effector cytokines, proved to be less effective therapeutically. It has been found that this approach may affect other key functions of inflammatory cytokines, for example, patients on long-term IL-1 β inhibitors may be more susceptible to infections (141). In addition, we need to be alert to the fact that inappropriate molecular therapy may not only lead to an increased susceptibility of the body to infectious and autoimmune diseases but also aggravate the disease by diminishing the body's anti-tumor immune response.

In clinical practice, resistance to immunotherapy is considered to be a great challenge. In-depth exploration of the mechanism of inflammasomes and immunotherapy drugs may help to solve this mystery. Currently, immune checkpoint inhibitors (ICIs) have been identified as effective anti-cancer therapies, such as anti-PD-1, in which the role of inflammasomes cannot be ignored. In the study on diffuse large B-cell lymphoma (DLBCL), Lu et al. found that NLRP3 inflammasome activation and significantly elevated IL-18 levels in DLBCL tissues promoted CD8⁺ T cell apoptosis by up-regulating programmed death ligand-1 (PD-L1), thereby exerting immunosuppressive effects (142). This process, to some extent, inhibits the tumor suppressive effect of anti-PD-1 drugs. Another showed that inhibition of the NLRP3 inflammasome significantly enhanced the efficacy of anti-PD-1 drugs (143). Huseni et al. found in a clinical study that interleukin-6 (IL-6) production due to inflammasome activation was associated with anti-PD-L1 resistance and that, compared with Atezolizumab(anti-PD-L1) alone, combined blockade of PD-L1 and IL-6 receptor (IL6R) significantly reduced drug resistance (144). In addition, the activation of NLRP3 inflammasome can exert immunosuppressive effects by promoting the expression of PD-L1 (145). These studies demonstrate a correlation between NLR inflammasome and adaptive resistance to anti-PD-1 checkpoint inhibitor immunotherapy in the treatment of cancer. Therefore, targeted antagonists against inflammasomes have the potential to become a new immunotherapy strategy.

Although many achievements have been made in the study of inflammasomes, many challenges remain, for example: (1) The activators of multiple inflammasomes are still unknown, and the signaling mechanisms activated by different inflammasomes need to be further explored (146). (2) Different inflammasomes play different roles in the same tissue, and the same inflammasomes play very distinct roles in different tissues (147), making the exploration of mechanisms extremely complicated. (3) Studies on the relevance of inflammasomes to tumors are limited and mostly focused on mouse models. (4) Some diseases involve the regulation of multiple NLR inflammasomes, and whether the

effect of the combination of these inflammasomes-targeted drugs is superior to the use of a single targeted drug, etc. These mysteries need more researchers to collaborate to unravel them one by one. Exploring the specificity of NLR inflammasomes in tissues or cells, thoroughly uncovering their biological roles and their pathogenic mechanisms in various stages of tumors, and finding possible therapeutic targets will contribute to the development of novel anti-cancer drugs.

Author contributions

QD, QG, JZ, and ZY make substantial contributions to the conception or design of the work. JZ, and ZY selected extracted relevant papers of this manuscript. QG wrote the manuscript. QD had primary responsibility for final content. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The emerging role of neutrophil extracellular traps in cancer: from lab to ward

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Neutrophil extracellular traps (NETs) are web-like structures derived from neutrophils, which typically consist of DNA, released from the nucleus or mitochondria, and decorated with histones and granule proteins. They are well known as an important structure in innate immunity to eliminate pathogenic bacteria, similar to neutrophils. Initially, NETs are reported to take part in the progression of inflammatory diseases; now, they have also been implicated in the progression of sterile inflammation such as autoimmune disease, diabetes, and cancer. In this review, we will describe the recent studies which have investigated the role of NETs in the development of cancer, especially metastasis. We also prescribe the strategies for targeting NETs in the multiple cancer types, which suggest that NETs are a promising treatment for cancer patients.

KEYWORDS

cancer, therapeutics, tumor microenvironment, neutrophil extracellular traps, metastasis (cancer metastasis)

1 Introduction

There are many ways to cure cancer, including surgery, chemotherapy, radiotherapy, and immunotherapy, however, recurrence and metastasis are still the main reason for the low survival rate of patients (1). The tumor microenvironment (TME) comprises all the non-cancerous host cells in the tumor, including fibroblasts, endothelial cells, neurons, adipocytes, adaptive, and innate immune cells, as well as its non-cellular components, including the extracellular matrix (ECM), and soluble products such as chemokines, cytokines, growth factors, and extracellular vesicles. The constant interactions between tumor cells and the TME play a decisive role in tumor initiation, progression, metastasis, and response to therapies (2, 3).

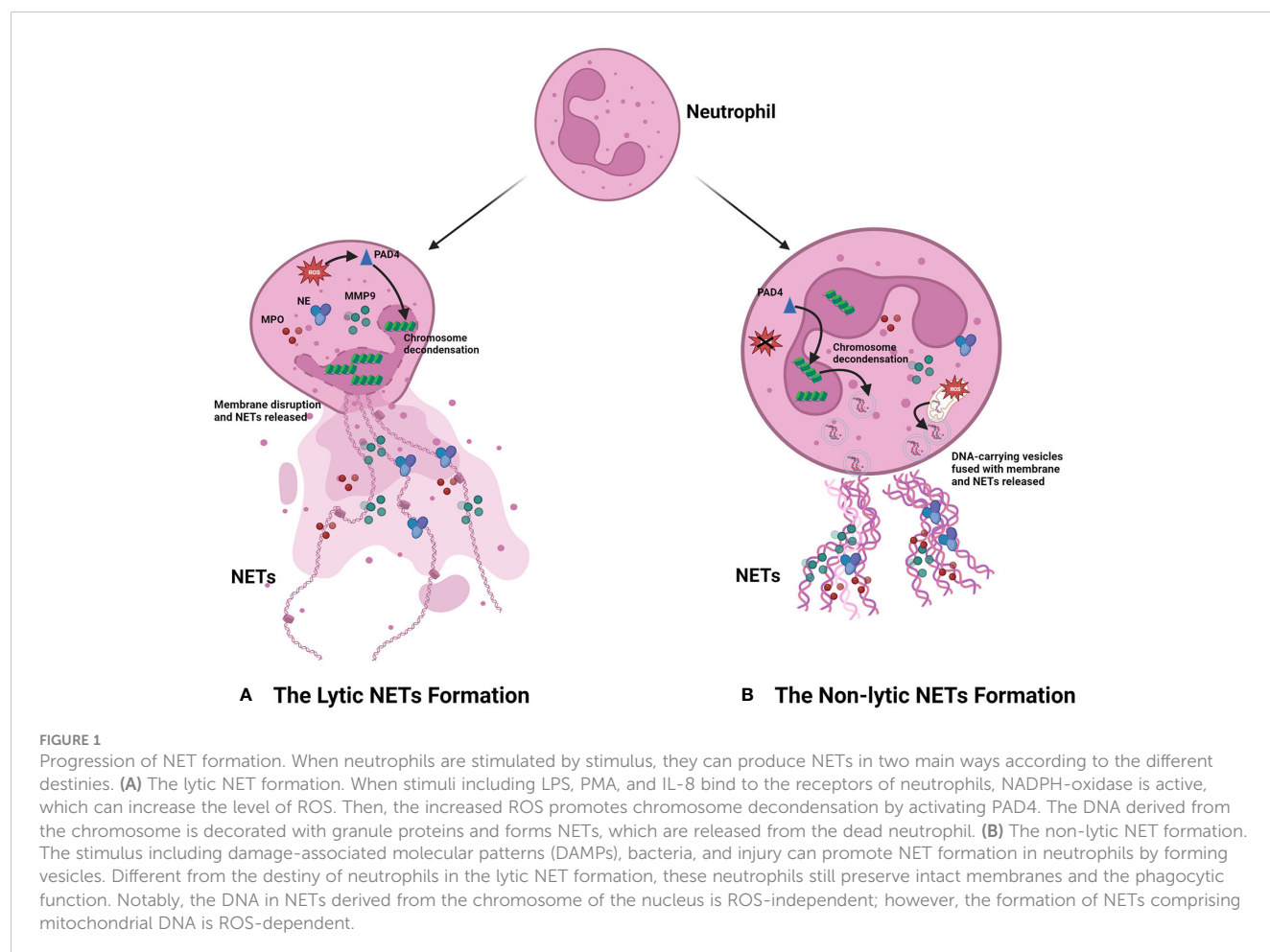
Neutrophils which take part in the pathogenesis of numerous diseases are the essential players in the early response against pathogens and during acute inflammation and play an important role in the regulation of innate and adaptive immune responses (4, 5). Recently, cancer-associated inflammation has been recognized as a hallmark of tumor biology (6). An inflammatory response to a tumor will contribute to cancer initiation and progression, allowing tumor cells to escape elimination by the immune system. Recent studies showed that neutrophils are an important component of the TME and have highlighted their importance in tumor progression and therapy (7–9). Due to the heterogeneity and plasticity of neutrophils, when receiving different external incentives, tumor-associated neutrophils (TAN) are polarized into antitumor and pro-tumor populations, which are named TAN-N1 and TAN-N2 (10). In 2004, Brinkmann et al. (11) first described that neutrophil extracellular traps (NETs), extracellular fibers released from neutrophils, consist of granule proteins and chromatin, bind Gram-positive and -negative bacteria, and are vital components of the innate response. The pathway of NET production has been described as a new form of cell death, NETosis, distinct from apoptosis and necrosis (12). In addition to their important role in defense capability, NETs also play an important role in the TME (13). In this review, we will not only introduce how the NETs are produced by neutrophils but describe the crosstalk between NETs

and tumor cells and the prognostic significance of NETs on cancer patients intensively.

2 An overview of nets

Previous research on neutrophils and their product, NETs, have mainly focused on inflammatory diseases, including sepsis and wound. In recent years, as studies involving neutrophils have intensified, it has been discovered that neutrophils and NETs are implicated in the progression of sterile inflammation including autoimmune disease, diabetes, and cancer (14–16).

NETs are unique net-like structure in the organism that originated from neutrophils. Like neutrophils, they act as the first defense of the organism against external stress, playing a part in removing foreign pathogens. The progression of NET formation was first described in 2004 (11); neutrophils are activated by external factors such as lipopolysaccharide (LPS) and phorbol myristate acetate (PMA) and then release intracellular DNA, histones, and granule proteins such as myeloperoxidase (MPO) and neutrophil elastase (NE) (17). These substances constitute the NETs in the extracellular compartment, and this special structure can be observed under the electron microscope. They play an important role in regulating the biological behavior of the tumor, especially tumor metastasis (18, 19).



The process of NET formation is known as “NETosis,” and there are two forms of NETosis based on whether the neutrophils lyse and die after the generation of NETs (Figure 1). The first is lytic NETosis, in which the neutrophil plasma membrane is cleaved and dies after the formation of NETs, and it lasts around 2–4 h. The other form is non-lytic NETosis, a new way different from lytic NETosis, described by Yipp et al. (20). During the pathway, neutrophils do not lyse and die after generating NETs; instead, they preserve the phagocytic function of normal neutrophils, and it starts within 1 h after stimulated by *Staphylococcus aureus* (21) and *Candida albicans* (22).

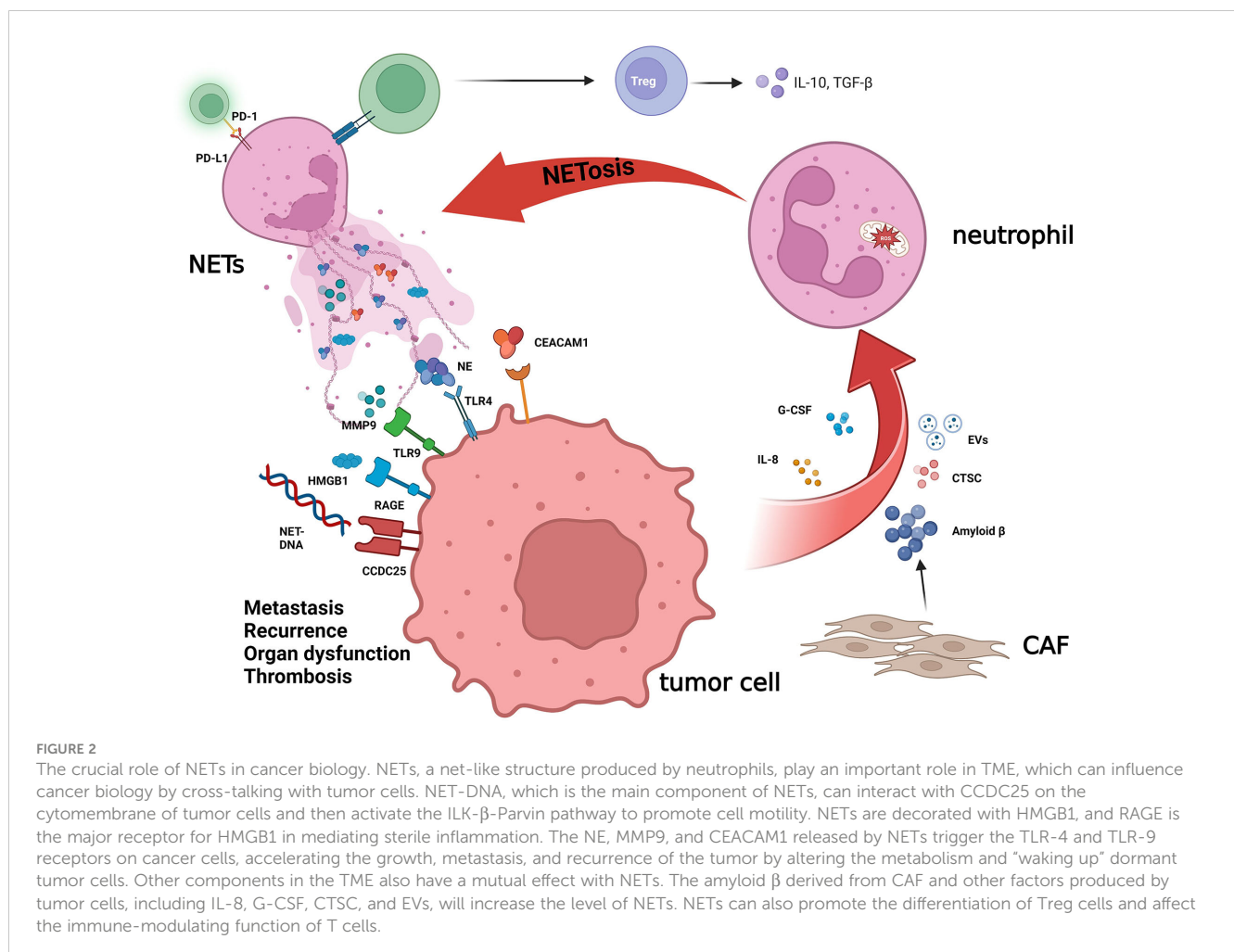
As the most extensively studied procedure for the formation of NETs, the stimuli of the lytic NETosis mainly include PMA, LPS, and interleukin-8 (IL-8), which, upon contact with neutrophils, initiate intracellular generation of reactive oxygen species (ROS) in an NADPH-dependent way, followed by activation of the peptidyl arginine deiminase 4 (PAD4), an essential enzyme in the NETosis process. PAD4 can facilitate chromatin decondensation, which allows DNA and histones to be excreted outside the cell and constitute the framework of NETs. It also activates a diverse range of granule proteins in neutrophils, such as NE and MPO, which bind to DNA extracellularly and collectively form the NETs (15). However, different from lytic NETosis, when external stimuli

provoke non-lytic NETosis, neutrophils can form DNA-carrying vesicles independent of ROS. These vesicles can then merge with the cytosolic membrane and deliver DNA to the extracellular space, after which it is combined with granule proteins from the neutrophils to form NETs (23, 24). In addition, the researchers have also discovered that the DNA within NETs can come to be originated not only from the nucleus but also from the mitochondria, an organelle containing low amounts of DNA (25–27). Between the two pathways, the most studied is the lytic NET formation, which is also mainly discussed in this review.

3 How nets promote tumor growth and metastasis

3.1 Crosstalk between tumor cells and NETs

The interaction between tumor cells and NETs includes several different ways (Figure 2). First, NETs can activate a variety of receptors and signal pathways associated with growth, and metastasis to shape the characteristics of the tumor. High mobility group box 1 (HMGB1), a protein widely distributed in



the body, has been discovered to have the pro-inflammatory function and becomes in recent years one of the popular targets of research in critical care medicine, and NETs also seem to enhance the malignancy of cancer (28), for the possible reason, just like Zhang et al. reported, that HMGB1 activates the nuclear factor-kappa B (NF- κ B) signaling pathway upon binding to the receptor for advanced glycation end products (RAGE) on the tumor cell surface and promotes tumor secretion of IL-8 (29, 30). In contrast, IL-8 recruits neutrophils and promotes the production of NETs, thereby creating a positive feedback, which also promotes colorectal cancer liver metastasis (31). The study by Tohme et al. (32) has indicated that NETs can promote HMGB1 production within tumor cells and activate TLR9-dependent pathways to promote tumor cell growth, metastasis, and invasive ability. Furthermore, the binding of NETs to tumor cells can also induce tumor cells to acquire resistance to death as well as enhanced invasiveness by activating the TLR4/9-COX2 pathway, and the use of DNase I in combination with the anti-inflammatory drugs can effectively reduce hepatocellular carcinoma metastasis (33). According to Albregues et al. (34), NETs can also “wake up” dormant tumor cells through metalloproteinase (MMP) and NE, facilitating metastasis and recurrence.

Similarly, tumor cells can also impact the formation of NETs by secreting some cytokines and proteins, the most investigated of which are IL-8 and granulocyte colony-stimulating factor (G-CSF) (30, 35–37). Xiao et al. (38) reported that cathepsin C (CTSC), the protease produced by tumor cells, can activate proteinase 3 (PR3) on the neutrophil membrane, to promote interleukin-1 β (IL-1 β) to process and NF- κ B to activate, which can upregulate interleukin-6 (IL-6) and CCL3, recruit neutrophils, and promote the production of ROS in neutrophils to induce NET formation. The extracellular vesicles (EVs) derived from the tumor are deemed to associate with the growth of cancer and modulate the TME and immune function (39, 40). The construction of a mouse model of breast cancer using 4T1 breast cancer cells reveals that 4T1-derived EVs promote the emergence of NETs and accelerate cancer-associated thrombosis in veins (41). Moreover, EVs derived from KRAS-mutated colorectal tumor cells can induce neutrophil recruitment and promote NET formation through IL-8 activation (42). Recently, Guimarães-Bastos et al. (43) revealed that EVs derived from melanoma cells can induce neutrophil chemotaxis, promote TAN polarization to TAN-N2, a pro-tumor population, and facilitate NET formation, thus contributing to tumor progression.

3.2 NETs chat with other components

In addition, as part of the tumor microenvironment, NETs can also interact with other tumor components. Compared with the normal, Zhang et al. (44) revealed that Th17 and interleukin-17 (IL-17) levels were significantly increased in pancreatic cancer and further found that the elevation of IL-17 induced neutrophil recruitment and NET production, which in turn had a suppressive effect on CD8⁺ T cells. Similarly, Kaltenmeier et al.

(45) observed that the immune function of T cells is significantly suppressed function in the TME with a high density of NETs. *In vitro*, they found that NETs also contained programmed cell death-ligand 1 (PD-L1), which inhibited T-cell function by combining with programmed cell death protein 1 (PD-1) on the T-cell surface, resulting in T-cell dysfunction and metabolic failure, and hence promoting tumor growth.

Cancer-associated fibroblasts (CAFs) are a common composition within the stroma, affecting tumor angiogenesis, stromal remodeling, and antitumor immunity and promoting tumor invasion (46–48). A recent study revealed the impact of CAFs on the formation of NETs (49); the amyloid- β produced by CAFs is involved in the induction of NETs by tumor cells through promoting intra-neutrophil ROS production and supports cancer progression, whereas NETs can also promote liver metastasis of pancreatic tumors by enhancing the migration ability of hepatic stellate cells and forming CAFs (50). DNA derived from NETs (NET-DNA) can also activate the stellate cells in the pancreas then forming fibrous stroma, promoting and enabling tumor proliferation by activating RAGE (51). CCDC25, a transmembrane protein, is a DNA receptor (52–54) and enhances cell motility by activating the ILK- β -Parvin pathway after binding to NET-DNA. Notably, they suggested that metastasis to the liver, but not other organs, was related to a higher level of NET-DNA in breast and colon cancer patients, implying that tumor metastases could be predicted by detecting NET-DNA content in the blood. In addition, Rayes et al. (55) prevented the metastasis of colon cancer by blocking carcinoembryonic Ag cell adhesion molecule 1 (CEACAM1), a component protein of NETs.

3.3 NETs promote metabolic reprogramming

Tumor cells can escape the immune clearance of the body through metabolic reprogramming. Variations in tumor metabolic pathways, including those favoring mitochondrial metabolism as well as oxidative phosphorylation, may allow tumor cells in the TME to cope better with stress (56). New studies show that NETs have an effect on the metabolism of tumor cells (57). NE in NETs can activate TLR-4 on the surface of tumor cells, leading to an accumulation of intracellular PGC1 α levels, which enhances the function of mitochondria and accelerates the growth of cancer.

In addition to directly affecting tumor cells' metabolism, NETs can also change the metabolism of immune cells. Non-alcoholic steatohepatitis (NASH) can be developed into hepatocellular carcinoma (HCC) with or without cirrhosis, Tsung et al. (58) found that those mice whose non-alcoholic steatohepatitis (NASH) was induced by a high-fat diet had greater NETs in the liver at an early stage, which could recruit the macrophages and promote the evolution of liver cancer. Inhibiting NET formation would not influence the development of a fatty liver but decrease the evolution of HCC. Subsequently, Wang et al. (59) used the FoxP3-DTR mouse model to simulate Treg cell clearance and discovered

that NETs could induce the development of NASH to HCC by promoting the oxidative phosphorylation of mitochondria within naïve CD4⁺ T cells and promoting their conversion to Treg cells, which also indicated that NETs could promote the connection between the innate and adaptive immune. Zenlander et al. (60), however, found no significant difference in the levels of NETs in patients with liver cancer that developed from cirrhosis compared with those with only cirrhosis. Also, tumor cells can promote NET production in a ROS-dependent pathway by inducing a transition from TAN to glycolytic and pentose phosphate metabolic pathways (61).

4 Clinical significance of nets

4.1 NETs in the tumor microenvironment

Recently, there has been growing research suggesting that NETs are important part of the tumor microenvironment after discharging from neutrophils and can influence not only the progression of the tumor but also the metastasis and therapy, especially the metastasis of cancer.

It was found that, compared with the normal tissues, the density of NETs was significantly higher in patients with breast, gastric, and lung cancers (62–64). Yang et al. (52) also found that NETs were abundant within liver metastases in cancer patients and the NET levels could be used for early prediction of liver metastases in breast cancer patients. Similarly, it has been reported that the peripheral blood neutrophil-to-lymphocyte ratio (NLR) correlates obviously with NETs in peripheral blood and the density of NETs tends to be higher in patients with lymph node metastases (65). Remarkably, the distribution of NETs was inconsistent in the tumor and its adjacent paraneoplastic tissues, with the highest density of NETs in the center of the tumor and the tendency for both the density of NETs and neutrophils to decrease from the center of the tumor to the stroma (65). It may be since neutrophils within the tumor are more likely to develop NETs (66); this suggests that intra-tumor NETs may be more capable of influencing the tumor. Nevertheless, it was found that cervical cancer with a high density of NETs in the stroma had a better prognosis. At the same time, the level of NET intratumor did not affect the patient's prognosis (67). Surendran et al. (68) developed a new three-dimensional (3D) tumor-immune microenvironment (TIME)-on-Chip device, which can simulate the TME *in vitro* to observe the neutrophil response during tumor cell proliferation and invasion. As a result, it was observed that NETs were formed when neutrophils came into contact with tumor cells and that NETs promoted tumor cell clustering and invasion into the stroma, which was more evident with NETs in the stroma. Therefore, it would be a promising field to explore the prognostic effects of NETs at different locations of the tumor.

Tumor metastasis is often the cause of poor prognosis for patients, including lymphatic metastasis and distant organ metastasis; the recurrence and metastasis of tumors can also be associated with NETs. First, epithelial–mesenchymal transition

(EMT), a common cause of metastasis, has been demonstrated to be modulated by NETs. Metastasis is always under the regulation of TME changes like inflammation, intravasation of angiogenesis, and cancerous cells, which is described as EMT. EMT enables epithelial cells to obtain a mesenchymal cell phenotype, accelerating the entry of tumor cells into the vascular system and leading to distant metastases (69, 70). Using purified NETs cocultured with colorectal cancer cell lines, Stehr et al. found that NETs could promote the cell motility of CRC cells (71), which was correlated with more mesenchymal biomarkers, and EMT increased the transcription factors while reducing the level of the epithelial biomarkers, such as E-cadherin (CDH1) and epithelial cell adhesion molecule (EPCAM). Similarly, the same results were observed in gastric cancer (63), pancreatic cancer (28), and lung cancer (72). NETs can promote EMT and metastasis in non-small cell lung cancer by inhibiting long non-coding RNA (lncRNA) MIR503HG expression and activating the NF- κ B pathway. NETs have also been shown to promote tumor cell entry into the circulatory system by downregulating intercellular tight-junction molecules (73–75). As an important component of the innate immune system, the complement system is significant in the process of tumor growth. Liu et al. (76) found that complement factor 5a (C5a), the downstream product of the C3b-catalyzed cleavage of C5, can recruit neutrophils, and membrane attack complex (MAC), a multiprotein containing several complement compositions, can promote NET formation by activating neutrophils that have contact with vascular endothelium. Then, NETs destroy the endothelial barrier and enhance vascular leakage, facilitating the entry of tumor cells into the blood and causing distant metastasis. Depleting the neutrophils or inhibiting the formation of MAC gives protection to the vascular endothelium and prevents the metastasis.

The alteration of the microenvironment in distant metastatic organs before tumor metastasis is known as pre-metastatic niche (PMN), and it helps to attract circulating tumor cells (CTCs) (77), thus promoting metastasis. There are six characteristics of PMN, namely, immunosuppression, angiogenesis/vascular permeability, inflammation, lymphangiogenesis, organotropism, and metabolic reprogramming. Zeng et al. (78) demonstrated that *in situ* breast cancer cells can enhance the level of hydroxy acid oxidase 1 (HAO1) in the lung, the rate-limiting enzyme of oxalate metabolism, and promote oxalate generation by activating the TLR3-IRF3 signaling pathway. Oxalate not only promotes the growth of metastatic tumor cells through the MAPK pathway but also activates NADPH oxidase, leading to increased ROS production, thus inducing the production of NETs and promoting the formation of PMN in the lung, making breast cancer more prone to metastasis to the lung. In addition, mesenchymal stem cells (MSCs) in the lung have potent pro-metastatic properties (79). Th2 cells in the lung induce C3 synthesis by MSCs through STAT6, which can induce neutrophil recruitment and NET formation to promote metastasis. By blocking the Th2-STAT6-C3-NET pathway, lung metastasis driven by MSCs was also attenuated. A particular type of neutrophil population was recently identified (80), tumor-associated aged neutrophils, whose

cell marker is CXCR4hiCD62Llo. In a constructed tumor metastasis model, tumor cells lead to the accumulation of aged neutrophils by disrupting neutrophil homeostasis and directly stimulating neutrophil aging regulated by angiotensin II. The aged neutrophils can release multiple metastasis-promoting factors like NETs and MMP9, and aged neutrophil permaturation can significantly increase liver metastasis of breast cancer and melanoma, which are mediated mainly by NETs. It has been observed that these cells are present not only early in the pre-metastatic microenvironment of the lung but also in the peripheral blood of patients (81). Aged neutrophils induce mitochondrial DNA release by sirtuin 1 (SIRT1), thereby inducing the formation of NETs, rather than the traditional Cit-Histone H3-dependent lytic NET formation, promoting breast cancer lung metastasis. Earlier research also found that the presence of NETs in the peritoneum as well as in the omentum contributes to PMN formation and promotes tumor metastasis (82, 83).

In addition to the above factors contributing to tumor metastasis, surgical operation and postoperative infection have been reported as risk factors for recurrence and metastasis in postoperative patients. For the majority of solid tumors, surgery is the preferred treatment to improve the prognosis of patients (84). However, the study found that whereas the operation removes the primary tumor, it is also deemed to promote the eruption of undetected microscopic lesions, increasing the possibility of recurrence and metastasis after surgery (85), and NETs also participated in the process (86). During the operation, with the destruction of the tumor and its associated blood vessels, some of the tumor cells can flow into the circulation system to form the CTCs, which act as “seeds” in the process of cancer recurrence and metastasis (87). In addition, tissue damage caused by surgery activates the immune and coagulation systems of the body, in which neutrophils, NETs (88), and platelets (89–91) can promote the tissue healing process, but they may also contribute to the spreading and metastasis of tumor. When CTCs enter the peripheral blood, they are rapidly coated by platelets to protect them from external stress and destruction by NK cells. Ren et al. (89) simulated the effects of surgery on the organism by constructing a model of liver ischemia–reperfusion injury (I/R) and showed that platelets were activated by local inflammation caused in I/R through the TLR4-ERK5 pathway and then bound to CTCs to form platelet-tumor cell clusters and that integrins could facilitate the connection of clusters and NETs and promote metastasis (92). In addition to the inflammatory response to tissue damage caused by surgery, postoperative infection, one of the common complications of the surgery, also promotes tumor recurrence and metastasis to some extent (93, 94). Postoperative peritoneal infection in gastric cancer induces NET formation and promotes gastric cancer invasion and metastasis by activating the TGF- β signaling pathway (95). Wang et al. have certificated that elevated LPS levels caused by a postoperative infection in colorectal cancer can induce NET production through the activation of TLR-9 and MAPK signaling pathways, which are closely associated with increased postoperative recurrence rates (96).

The link between cancer and thrombosis has been discovered for decades (97, 98), whereas increased levels of blood clotting factors, tissue factor, and activation of fibrous protein has been described as the mechanism. However, the exact mechanism causing this change is not known. Fuchs et al. (99) revealed that NETs can promote platelet adhesion, aggregation, and activation in the vasculature and induce thrombosis. In the research of Demers et al. (100) in 2012, it was first described that induction of NETs by G-CSF, which was derived from tumor cells, could promote coagulation in tumor patients, leading to cancer-associated thrombosis formation. Subsequent studies (101–104) also demonstrated that neutrophils and NETs contribute to platelet activation and tissue factor synthesis, leading to the formation of venous thrombosis in cancer patients. However, it has been shown in other investigations that NETs only affect the formation of atherothrombosis in tumor patients and do not affect venous thrombosis (41, 105). Therefore, whether NETs affect venous thrombosis in cancer patients and the specific mechanisms involved need further investigation. Other than promoting the formation of cancer-associated thrombosis, some researchers have recently suggested that NETs may also affect the myocardium (106). Using mice with breast cancer, they found a correlation between myocardial dysfunction and NETs in mice, and inhibiting the formation of NETs improved the inflammatory response of the myocardium and decreased the level of biomarkers.

4.2 NETs in cancer therapy

NETs have been previously investigated in sepsis and other inflammatory diseases and can be a more promising target for cancer therapy based on their proliferation-promoting and metastatic effects on tumors as well as their impact on the TME. The current therapeutic approach to NETs consists of two main aspects, inhibition of NET formation or destruction of formed NETs. NET-DNA from the nucleus and mitochondria, which is the vital composition of NETs, can be hydrolyzed by DNase I. NET-DNA can accelerate the growth and metastasis of cancer after binding to CCDC25, and destroying NETs by using DNase I is frequently used in current trials (107, 108). Many recent studies proved that heparin can promote the degradation of NETs by detaching histones from the NET-DNA skeleton (99); the use of low molecular heparin can hinder the formation of NETs induced by PMA (109). However, a recent study found that heparin can induce NET formation *in vitro* (110). This suggests that further research is expected to confirm whether low molecular heparin can be used to degrade NETs and by what mechanism.

Interfering with the formation of NETs can be a positive strategy instead of degrading the formed NETs by suppressing the compositions crucial for the NETs, such as PAD4, NE, or MPO (Table 1). Chromatin densification is the most pivotal process in the formation of NETs, and it is dependent on the existence of PAD4 (57). Lewis et al. (114) have recommended two inhibitors of the PAD4, especially GSK484, which can suppress disease by destroying

TABLE 1 Targeting NETs in multiple cancer types.

Major impact	Targets	Inhibitors	Cancer type	Reference
Inhibiting NET formation	NADPH	Kaempferol	Breast cancer	(111)
	PAD4	BMS-P5	Multiple myeloma	(112)
		CI-amidine	Chronic myeloid leukemia	(113)
		GSK484	Cancer-associated kidney injury	(114, 115)
		JB1-589	Lung and colon cancer	(116)
Accelerating NET destruction	NET-DNA	DNase	Breast cancer; colorectal cancer; hepatocellular carcinoma; cancer-associated thrombosis	(73, 107, 117, 118)
	NE	GSDMD	Melanoma	(119)
		Sivelestat	Lung and colon cancer; gastric cancer	(18, 120)
		GW311616A	Large B-cell lymphoma	(121)
Blocking the pathway	NF- κ B	NBD peptide	Breast cancer	(30)
	TLR-9	Hydroxychloroquine	HCC; pancreatic cancer	(33, 102)
	DDR1	7th benzamide	Pancreatic cancer	(122)

NETs. NE and MPO are the critical compositions of NETs, and mouse models lacking NE have been utilized to study the effects of decreased NETs on cancer metastasis (18, 86) and sepsis (123). With regard to MPO, which is usually viewed as a marker of NETs, it can also influence the NETs. Mice treated with MPO inhibitors could not form NETs and are always utilized to observe the impacts of NETs on cancer. Based on the increasing research on NETs and tumors, specific blockade of the interplay has become a new therapeutic option. Blocking NETs or tumor cell-derived factors, including IL-8, IL-17, and their receptors, has been shown to affect the biological behavior of cancer (44, 124–126).

Furthermore, NETs have been demonstrated to play a role in tumor treatment resistance, including chemotherapy resistance (127), immunotherapy resistance (44, 128, 129), and radiation therapy resistance (130). NETs were observed in radiation-resistant bladder cancer patients compared with the radiation-sensitive patients, and inhibiting HMGB1 and NETs significantly improved the outcome of radiation therapy (130). The TME has a vital role in cancer immunity and probably helps to inhibit the effects of immune checkpoint inhibitors and other new immunotherapies in terminal cancer patients. Zhang et al. (129) used DNase I to degrade NETs that could decrease the resistance to anti-PD-1 therapy in a CRC model. However, a study by Liu et al. (131) indicated that NETs show a novel immunomodulatory role in *Bacillus Calmette-Guerin* (BCG) immunotherapy. Tumor cells activated by BCG can induce NETs through their production of IL-8 and TNF α , and these NETs help to recruit T cells and macrophages and repair damaged tissue, inducing tumor cell apoptosis and cell cycle arrest.

5 Conclusion

Despite the multiple treatment options available for cancer, recurrence, and metastasis are currently still the most common cause of patient death. With intensive research in recent years, the TME has been recognized as the main influencing factor of tumor behavior. The cellular debris produced by apoptosis and necrosis of tumor cells leads to local inflammatory reactions, activating the innate immune response and recruiting neutrophils. NETosis is a vital way by which neutrophils function, but research on the impacts of NETs on tumor cells is still in its infancy. Although a few studies have suggested that NETs have some antitumor effects, it is clear that NETs play a role in promoting the proliferation, invasion, and metastasis of tumors, and even the effectiveness of chemotherapy, radiotherapy, and immunotherapy on cancer patients is related to NETs.

There are mounts of mechanisms underlying NET-dependent tumor progression and metastasis. As reviewed herein, NETs and tumor cells in the TME have been shown to interact through the production of multiple factors, proteins, and their receptors. All results of different studies listed in this review imply the need for further studies about the interaction between NETs and tumors. Although there are some indications that NETs are associated with prognosis in cancer patients, there is still a lack of relevant clinical trials. In addition, it should be noted that neutrophils and NETs are important components of innate immunity, and inhibition of NET formation or destruction of formed NETs may affect neutrophils and reduce pathogenic clearance. Hence, the development of therapies that can accurately target NETs within the tumor

without adversely affecting immune function is necessary. Taken together, the emerging role of NETs in cancer diagnosis, growth, invasion, metastasis, and therapy should attract enough attention.

Author contributions

WZ designed and wrote the manuscript and drafted the figures, QW searched relevant literature. XS and JD supervised this review. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

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Advanced lung cancer inflammation index is associated with long-term cardiovascular death in hypertensive patients: national health and nutrition examination study, 1999–2018

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Background: Hypertension is one of the main causes of cardiovascular death. Inflammation was considered influential factors of cardiovascular (CVD) death in patients with hypertension. Advanced lung cancer inflammation index (ALI) is an index to assess inflammation, few studies have investigated the relationship between advanced lung cancer inflammation index and cardiovascular death in hypertensive patients.

Objective: The aim of this study was to investigate the association between advanced lung cancer inflammation index and long-term cardiovascular death in hypertensive patients.

Method: Data from the National Health and Nutrition Examination Survey (NHANES) 1999–2018 with mortality follow-up through 31 December 2019 were analyzed. Advanced lung cancer inflammation index was calculated as BMI (kg/m²) × serum albumin level (g/dL)/neutrophil to lymphocyte ratio (NLR). A total of 20,517 participants were evaluated. Patients were divided into three groups based on tertiles of advanced lung cancer inflammation index as follows: T1 (*n* = 6,839), T2 (*n* = 6,839), and T3 (*n* = 6,839) groups. The relationship between advanced lung cancer inflammation index and long-term cardiovascular death was assessed by survival curves and Cox regression analysis based on the NHANES recommended weights.

Results: The median advanced lung cancer inflammation index value in this study was 61.9 [44.4, 84.6]. After full adjustment, the T2 group (hazard ratio [HR]: 0.59, 95% confidence interval [CI]: 0.50–0.69; *p* < 0.001) and T3 group (HR: 0.48, 95% CI: 0.39–0.58; *p* < 0.001) were found to have a significantly lower risk of cardiovascular death compared to the T1 group.

Conclusion: High levels of advanced lung cancer inflammation index were associated with reduced risk of cardiovascular death in hypertensive patients.

KEYWORDS

hypertension, inflammation, cardiovascular death, advanced lung cancer inflammation index, NHANES

Introduction

Hypertension is a chronic disease that can be effectively intervened, and it is one of the primary causes of death from cardiovascular disease (CVD) (Shin et al., 2019). In fact, approximately half of all CVD deaths were related to hypertension (Kearney et al., 2005; Lim et al., 2012). Despite significant advancements in hypertension treatment and management, its impact on CVD mortality continues to rise worldwide. (Mills et al., 2016; Das, 2017).

Past research has revealed that inflammation plays a crucial role in the onset and progression of hypertension. Chronic inflammation can significantly elevate the risk of death from CVD. (Virdis et al., 2014; Boos et al., 2021). The majority of present-day studies that employ inflammatory markers to evaluate the prognosis of hypertension only utilize individual inflammatory markers (Engström et al., 2006; Cortez et al., 2016; Sun et al., 2017). Nevertheless, inflammation can result in decreased albumin and weight loss (Lennie, 1998; Sheinenzon et al., 2021). and relying on a single inflammatory marker may not provide sufficient precision to evaluate the prognosis of patients with hypertension.

The advanced lung cancer inflammation index, an index that combines body weight, albumin and neutrophil to lymphocyte ratio (NLR), was originally used to assess systemic inflammation levels in cancer patients (Jafri et al., 2013). In previous studies, ALI showed good efficacy in assessing inflammatory status in coronary artery disease and heart failure patients, and was associated with prognosis in these patients (Maeda et al., 2020; Fan et al., 2021). Given that hypertension was believed to have a connection with inflammation, we utilized the ALI to evaluate the inflammatory status among patients with hypertension, and investigated its correlation with death from CVD in hypertensive patients.

The purpose of this study was to investigate the relationship between ALI and the risk of CVD death in patients with hypertension and to provide some reference for the treatment and management of hypertensive patients.

Materials and methods

Study population

NHANES is a nationally representative cross-sectional survey recursively conducted in the United States by the National Center for Health Statistics. NHANES is based on a stratified multistage random sampling design. A retrospective analysis was performed using publicly available data from NHANES from 1999 to 2018.

In NHANES 1999–2018, our analysis was limited to 23,765 participants aged 18 years and older with hypertension. Hypertension was defined as an affirmative answer by participants to the question “Have you ever been told by a doctor or other health professional that you have hypertension, also called high blood pressure?” In addition, participants with systolic blood pressure (SBP) ≥ 140 mmHg or/and diastolic blood

pressure (DBP) ≥ 90 mmHg were defined as having hypertension. If the participants had their blood pressure measured more than one time, their average blood pressure was used to determine whether the patient had hypertension (Unger et al., 2020). In addition, participants who were receiving antihypertensive medications are also considered to have hypertension. Of these participants, 3,222 people who lacked body mass index (BMI), albumin, neutrophil, and lymphocyte data were excluded. In addition, 26 participants who were lost follow-up were excluded. Ultimately, a total of 20,517 participants were included in this cohort study (Figure 1).

Calculation of ALI

ALI was calculated as BMI (kg/m^2) \times serum albumin level (g/dL)/neutrophil-to-lymphocyte ratio (NLR). Patients were divided into three groups based on the tertiles of ALI: T1 group (≤ 50.0), T2 group (>50.0 and ≤ 77.0), and T3 group (>77.0).

Primary outcome

The primary outcome was CVD death. Cause of death was categorized using the International Classification of Diseases 10th edition (ICD-10). Cardiovascular mortality was categorized using ICD-10 codes I00–I078. For participants in NHANES 1999–2018, mortality follow-up data was available through 31 December 2019.

Definitions of variables of interest

Age, sex, race, smoking status and drink status were self-reported by participants. Participants who were currently taking calcium channel blockers (CCB), diuretics, beta blockers, and angiotensin converting enzyme inhibitors (ACEI)/angiotensin II receptor blockers (ARB) were considered to be taking antihypertensive drugs. Laboratory measurements, such as creatinine (Cr), triglyceride (TG), total cholesterol (TC), blood glucose (Glu), albumin, neutrophil counts, and lymphocyte counts, were collected using automated hematological analysis equipment. Detailed procedures for obtaining laboratory measurements were provided in a document on the website of the National Center for Health Statistics. The Healthy Eating Index (HEI-2015) was calculated based on the patient's total nutrient intake on the first day. Diagnosis of comorbidities was based on an affirmative response to the question “Has a doctor or other health professional ever told you that you had chronic heart failure (CHF), chronic heart disease (CHD), diabetes mellitus (DM), stroke, or cancer?”

Statistical analyses

We used the NHANES recommended weights to calculate the weights for specific groups. Continuous variables were expressed as

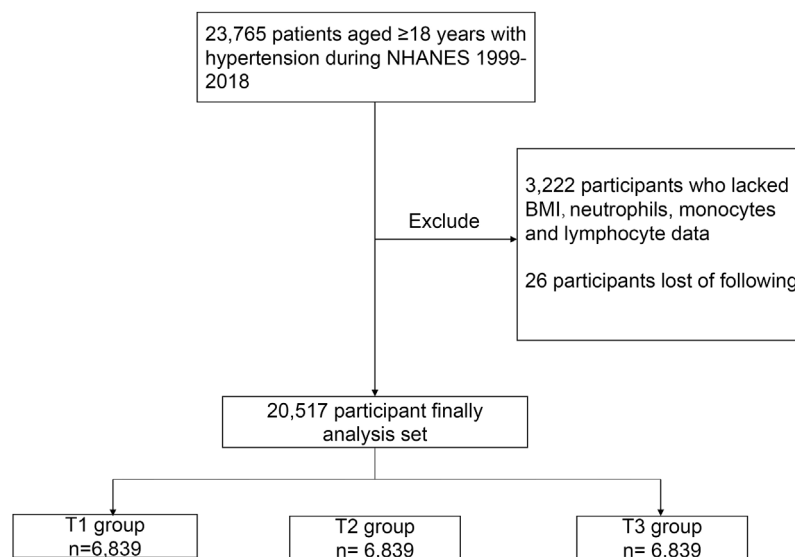


FIGURE 1
Flowchart of the study design.

the mean \pm standard deviation. Variables that do not conform to the normal distribution are represented by the median (25th percentile, 75th percentile). Categorical variables were presented as counts (percentages). Baseline characteristics between the three groups were compared using an analysis of variance (ANOVA) for continuous variables and an χ^2 test for categorical variables.

To evaluate the association between ALI and long-term CVD death, we used Kaplan-Meier and Cox regression analyses. Both estimates and probabilities were based on weights recommended by NHANES. Model 1 was a crude model unadjusted for potential confounders. Model 2 was adjusted for age and sex. Model 3 was fully adjusted for potential confounders. Furthermore, we explored the relationship between ALI and CVD mortality in different subgroups including age, sex, BMI, antihypertensive drug and DM. Restricted regression cubic splines were used to explore the potential non-linear relationship between ALI and CVD death in hypertensive patients. COX regression analysis was performed on the variables required for ALI calculation.

All data analyses were performed by using the Survey package in R software (version 4.0.4; R Foundation for Statistical Computing, Vienna, Austria). A two-sided p -value <0.05 indicated significance for all analyses.

Results

Participant characteristics

Among all 20,517 participants eligible for the study, the average age was 54.8 ± 0.2 years. The distribution of ALI is shown in [Supplementary Figure S1](#). Approximately half ($n = 10,379$, 50.9%) were female. Patients were divided into three groups based on the tertiles of ALI: T1 group ($n = 6,839$), T2 group ($n = 6,839$), and T3 group ($n = 6,839$). The median ALI of the

T2 [62.5 (IQR: 56.4 – 69.1)] and T3 [97.8 (IQR: 85.6 – 119.7)] groups was found to be higher than that of the T1 group [38.0 (IQR: 29.9 – 44.4)]. Participants in the group with higher ALI were younger (T1 group: 61.1 ± 0.3 vs. T2 group: 55.8 ± 0.3 vs. T3 group: 53.5 ± 0.3 years) and had higher BMI (T1 group: 27.7 ± 0.1 vs. T2 group: 30.9 ± 0.1 vs. T3 group: 33.8 ± 0.1 kg/m²) and were more likely to be female (T1 group: 49.8% vs. T2 group: 49.6% vs. T3 group: 53.6%). In the group with higher ALI, participants have lower Cr (T1 group: 89.61 ± 0.99 vs. T2 group: 81.30 ± 0.43 vs. T3 group: 79.41 ± 0.39 μ mol/L) and Healthy Eating Index–2015 (HEI–2015) (T1 group: 51.3 ± 0.2 vs. T2 group: 50.8 ± 0.3 vs. T3 group: 50.3 ± 0.3). With increased ALI, the proportion of smokers gradually decreased (T1 group: 54.3% vs. T2 group: 50.4% vs. T3 group: 46.7%) and was less likely to be combined with stroke (T1 group: 7.8% vs. T2 group: 5.2% vs. T3 group: 4.4%), CHD (T1 group: 10.2% vs. T2 group: 6.5% vs. T3 group: 5.3%), CHF (T1 group: 7.0% vs. T2 group: 4.2% vs. T3 group: 3.9%) and cancer (T1 group: 19.7% vs. T2 group: 13.4% vs. T3 group: 10.9%). There was no statistical difference in DM among the three groups. In addition, the group with higher ALI levels had lower rates of CCB (T1 group: 19.4% vs. T2 group: 16.9% vs. T3 group: 14.9%) and β -block use (T1 group: 26.8% vs. T2 group: 21.8% vs. T3 group: 20.8%). There was no statistical difference in the number of people using ACEI/ARB and diuretics. More data on the baseline characteristics of study population are detailed in [Table 1](#).

ALI and CVD mortality

Out of all the participants, 1,453 (6.5%) individuals died due to CVD, with 739 (3.3%) in the T1 group, 424 (1.9%) in the T2 group, and 290 (1.3%) in the T3 group. Kaplan-Meier survival analysis curves revealed that the groups with higher ALI had lower mortality rates from CVD (P -log rank <0.001 , [Figure 2](#)). The results of

TABLE 1 Baseline characteristics of the study population (weighted).

	Overall (n = 20,517)	T1 group (n = 6,839)	T2 group (n = 6,839)	T3 group (n = 6,839)	p-value
ALI, mean [IQR:25%–75%]	61.9 [44.4, 84.6]	38.0 [29.9, 44.4]	62.5 [56.4, 69.1]	97.8 [85.6, 119.7]	<0.001
Age, (years), mean (SE)	56.8 ± 0.2	61.1 ± 0.3	55.8 ± 0.3	53.5 ± 0.3	<0.001
Female, n (%)	10,379 (50.9)	3,188 (49.8)	3,448 (49.6)	3,43 (53.6)	<0.001
Race, n (%)					<0.001
Mexican American	2,990 (5.6)	880 (4.6)	1,170 (6.4)	940 (5.9)	
Non-Hispanic Black	5,021 (12.8)	1,042 (7.2)	1,374 (10.0)	2,605 (21.7)	
Non-Hispanic White	9,459 (71.1)	3,966 (79.2)	3,199 (72.4)	2,294 (61.2)	
Other Hispanic	1,515 (4.4)	457 (3.6)	546 (4.9)	512 (4.7)	
Other Race	1,532 (6.0)	494 (5.4)	550 (6.2)	488 (6.4)	
BMI, (kg/m ²), mean (SE)	30.8 ± 0.1	27.7 ± 0.1	30.9 ± 0.1	33.8 ± 0.1	<0.001
SBP, (mmHg), mean (SE)	134.3 ± 0.2	135.5 ± 0.4	133.6 ± 0.4	133.6 ± 0.3	<0.001
DBP, (mmHg), mean (SE)	74.2 ± 0.2	72.2 ± 0.3	74.6 ± 0.3	75.8 ± 0.3	<0.001
Neutrophil, (K/μL), mean (SE)	4.44 ± 0.02	5.36 ± 0.03	4.41 ± 0.03	3.51 ± 0.02	<0.001
Lymphocyte, (K/μL), mean (SE)	2.14 ± 0.02	1.64 ± 0.01	2.12 ± 0.01	2.69 ± 0.04	<0.001
Albumin, (g/dL), mean (SE)	4.23 ± 0.01	4.18 ± 0.01	4.25 ± 0.01	4.26 ± 0.01	<0.001
Cr, (μmol/L), mean (SE)	83.47 ± 0.42	89.61 ± 0.99	81.30 ± 0.43	79.41 ± 0.39	<0.001
TG, mmol/L, mean (SE)	1.92 (0.02)	1.77 (0.02)	1.99 (0.03)	2.02 (0.03)	<0.001
TC, mmol/L, mean (SE)	5.14 (0.014)	5.03 (0.021)	5.172 (0.020)	5.23 (0.02)	<0.001
Glu, mmol/L, mean (SE)	5.91 (0.02)	5.953 (0.03)	5.932 (0.04)	5.826 (0.03)	<0.001
HEI-2015, mean (SE)	50.8 ± 0.2	51.3 ± 0.2	50.8 ± 0.3	50.3 ± 0.3	0.004
Smoke status, n (%)	10,170 (50.5)	3,699 (54.3)	3,351 (50.4)	3,120 (46.7)	<0.001
Drink status, n (%)	11,275 (67.8)	3,683 (67.1)	3,812 (69.3)	3,780 (66.9)	0.082
DM, n (%)	5,966 (23.8)	1,938 (23.8)	1,973 (23.1)	2,055 (24.7)	0.221
CHF, n (%)	1,276 (5.1)	584 (7.0)	371 (4.2)	321 (3.9)	<0.001
CHD, n (%)	1,608 (7.3)	721 (10.2)	522 (6.5)	365 (5.3)	<0.001
Stroke, n (%)	1,452 (5.8)	640 (7.8)	451 (5.2)	361 (4.4)	<0.001
Cancer, n (%)	2,805 (14.7)	1,307 (19.7)	827 (13.4)	671 (10.9)	<0.001
CCB, n (%)	4,196 (20.5)	1,516 (19.4)	1,408 (16.9)	1,272 (14.9)	<0.001
Beta blockers, n (%)	4,921 (24.0)	1,899 (26.8)	1,578 (21.8)	1,444 (20.8)	<0.001
ACEI/ARB, n (%)	8,436 (41.1)	2,867 (41.1)	2,823 (39.3)	2,746 (39.2)	0.481
Diuretics, n (%)	6,028 (29.4)	2,021 (28.5)	1,970 (27.2)	2,037 (27.9)	0.796
Antihypertensive drugs, n (%)	13,023 (63.5)	4,517 (64.5)	4,261 (59.5)	4,245 (59.8)	<0.001
CVD death, n (%)	1,453 (6.5)	739 (3.3)	424 (1.9)	290 (1.3)	<0.001

Note: T1 group (ALI ≤ 50.00), T2 group (ALI > 50.00, and ≤77.00), T3 group (ALI > 77.00).

The average number of drinks consumed per day over the past 12 months.

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALB, albumin; Cr, creatinine; TG, triglyceride; TC, total cholesterol; HEI-2015, healthy Eating Index-2015; DM, diabetes mellitus; CHF, congestive heart failure; CHD, coronary heart disease; CCB, calcium channel blockers; ACEI, angiotensin converting enzyme inhibitors; ARB, Angiotensin II, receptor blockers.

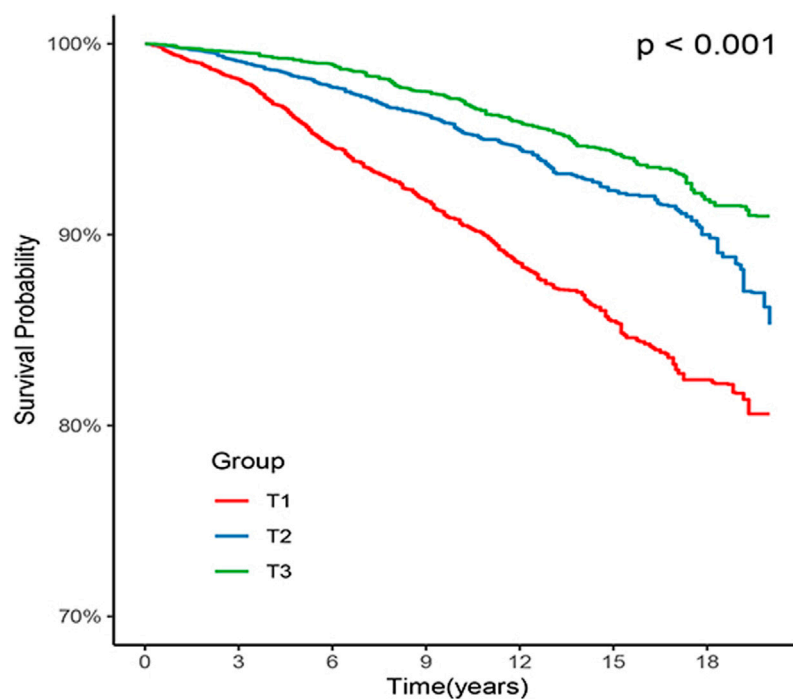


FIGURE 2

Kaplan-Meier survival estimates for long-term cardiovascular mortality (weighted).

TABLE 2 Associations between ALI and cardiovascular mortality in NHANES 1999–2018 followed through 2019.

Variable	Model 1			Model 2			Model 3		
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
Continuous variables									
ALI per 10 U	0.84	0.81–0.87	<0.001	0.92	0.90–0.94	<0.001	0.89	0.87–0.92	<0.001
Tripartite variable									
T1 group		Ref			Ref			Ref	
T2 group	0.47	0.41–0.53	<0.001	0.64	0.56–0.72	<0.001	0.59	0.50–0.69	<0.001
T3 group	0.32	0.27–0.38	<0.001	0.56	0.48–0.65	<0.001	0.48	0.39–0.58	<0.001

Model 1: No adjusted.

Model 2: Adjusted by age, gender.

Model 3: Adjusted by age, gender, race/ethnicity, smoke, drink, BMI, cr, TG, TC, glu, CHF, CHD, DM, stroke, antihypertensive drugs, cancer, HEI-2015, DBP, SBP.

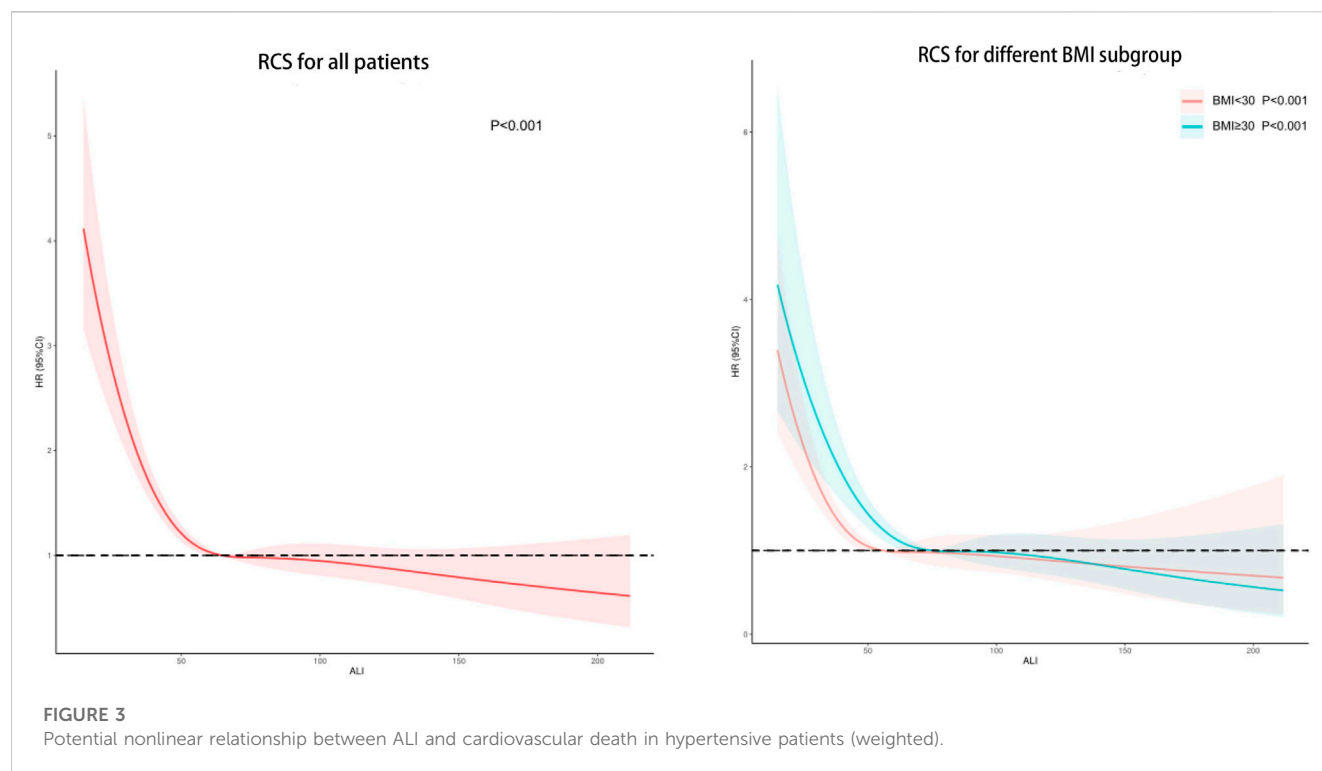
univariate Cox proportional hazard analysis showed that the risk of death from CVD decreased by 16% for each 10 unit increase in ALI (95% confidence interval (CI): 0.81–0.87; $p < 0.001$). In comparison to the T1 group, both the T2 (hazard ratio (HR): 0.47, 95% CI: 0.41–0.53; $p < 0.001$) and T3 (HR: 0.32, 95% CI: 0.27–0.38; $p < 0.001$) groups had a lower risk of death from CVD. After adjusting for potential confounders including age, sex, ethnicity, smoking, drinking, Cr, TG, TC, Glu, CHF, CHD, DM, stroke, antihypertensive drugs, cancer, HEI-2015, DBP, and SBP, the risk of CVD death decreased by 11% (95% CI: 0.87–0.92; $p < 0.001$) for each 10 unit increase in ALI. The T2 (HR: 0.59, 95% CI: 0.50–0.69; $p < 0.001$) and T3 (HR: 0.48, 95% CI: 0.39–0.58; $p < 0.001$) groups had a lower risk of CVD death compared to the T1 group (Table 2).

Restricted regression cubic spline

The results of the restricted RCS analysis indicated no non-linear relationship between ALI and CVD death in hypertensive patients, and low levels of ALI were associated with an increased risk of CVD death in this population. Stratification by BMI did not significantly alter the results. (Figure 3).

Subgroup analysis

When participants were stratified by age (P for interaction = 0.531), sex (P for interaction = 0.930), BMI (P for interaction =



0.361), DM (P for interaction = 0.317) and antihypertensive drugs (P for interaction = 0.269) the association between ALI and CVD mortality did not change. With the increased of ALI, the risk of CVD death decreased. The results of a stratified analysis by drug were consistent with the main effect (Figure 4).

Supplementary analysis

Among the items required for ALI calculations, alb and NLR were associated with the risk of CVD death in hypertensive patients after full adjustment for confounding variables (Supplementary Table S1). After grouping by BMI quintile, groups Q2, Q3, and Q4 had a lower risk of CVD death compared to group Q1. The Q5 group was not statistically significant (Supplementary Table S2). In addition, the results of a subgroup analysis of ALI showed that elevated ALI was associated with a reduced risk of CVD death in hypertensive patients in the subgroup with $ALI \leq 60$. p values were not statistically significant in the subgroup with $ALI > 60$, despite a downward trend in CVD death risk (Supplementary Table S3).

Discussion

This cohort study conducted in the United States utilized ALI to evaluate the inflammatory status of hypertensive patients. The findings indicated that patients with higher ALI had a decreased risk of CVD death, even after controlling for various confounding factors. This association remained consistent across different age groups, genders, BMI categories, and DM status. These results suggest that lower levels of inflammation were associated with a lower risk of CVD death in individuals with hypertension.

Hypertension was considered to be disease associated with inflammation (Steven et al., 2019). The chronic, low-grade inflammatory state in hypertensive patients can contribute to vascular remodeling, leading to vascular fibrosis and an increased risk of CVD-related mortality. (Intengan and Schiffrin, 2001). In a prospective study, hypertension patients with higher levels of inflammation had a 2-fold increased risk of all-cause death and a 1.8-fold increased risk of CVD (Cortez et al., 2016). The findings of these studies all suggest that inflammation was detrimental prognostic factors in patients with hypertension, which was in line with our results. However, it was worth noting that prior research has typically relied on a single inflammatory marker to evaluate the prognosis of hypertensive patients (Engström et al., 2006; Cortez et al., 2016; Sun et al., 2017). As mentioned in the introduction, inflammation can accelerate protein breakdown, resulting in a reduction in albumin levels. Moreover, inflammation can also induce insulin resistance, diminish appetite, and hinder the absorption of nutrients, ultimately leading to weight loss (Yoon et al., 2016; Guescini et al., 2017; Merker et al., 2020; Aldhwayan et al., 2022; Dou et al., 2022). Hence, relying solely on a single inflammatory marker to evaluate the mortality risk in patients with hypertension may not provide sufficiently accurate results.

ALI was calculated as $BMI (kg/m^2) \times serum\ albumin\ level (g/dL)/NLR$. Due to the fact that inflammation often results in hypoproteinemia and decreased BMI, previous studies have combined these two parameters with inflammatory markers to comprehensively evaluate the systemic levels of inflammation in cancer patients. It has been reported that lung cancer patients with higher ALI have a reduced risk of death (Jafri et al., 2013). In non-cancer populations, the effectiveness of ALI has also been

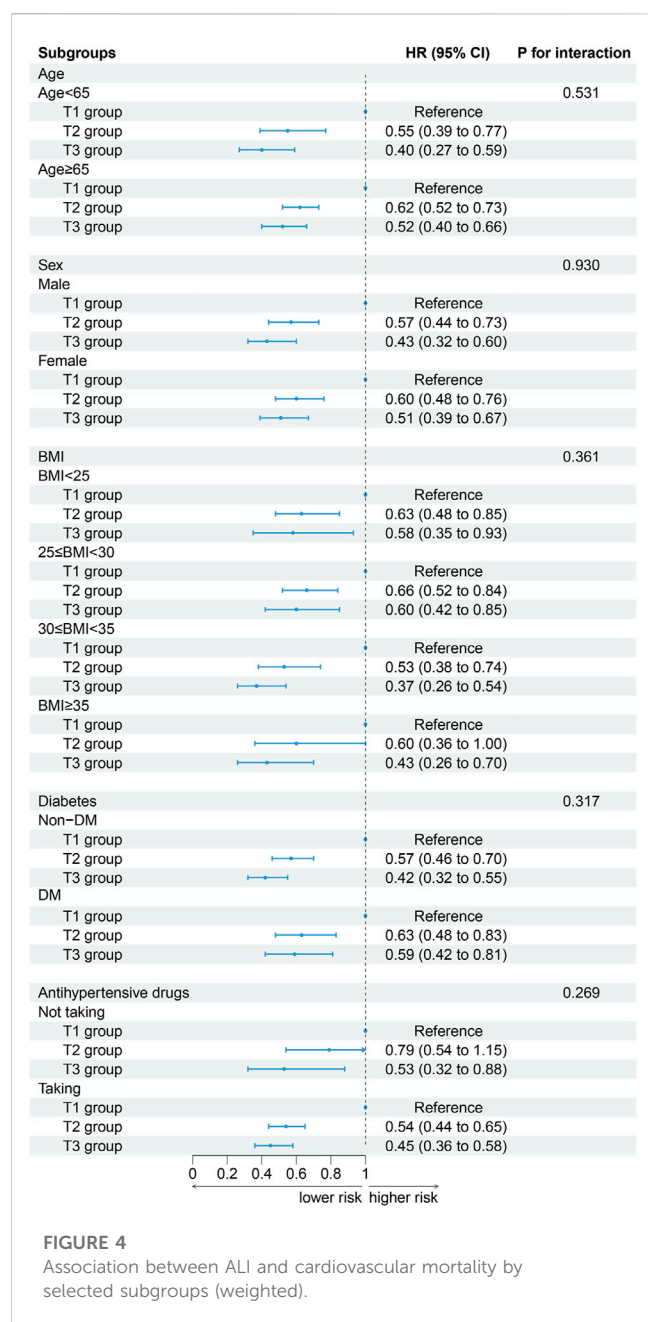


FIGURE 4
Association between ALI and cardiovascular mortality by selected subgroups (weighted).

demonstrated. Several studies have shown that low ALI is associated with increased risk of coronary artery calcification, readmissions and death in patients with heart failure (Maeda et al., 2020; Fan et al., 2021; Yuan et al., 2022). However, few studies have utilized ALI to evaluate the risk of CVD death in patients with hypertension. Our findings demonstrate that higher ALI levels are associated with a reduced risk of CVD death in patients with hypertension. Moreover, the results remained consistent when stratified by age, sex, BMI, antihypertensive drug use, and DM.

As high BMI is a well-established risk factor for CVD mortality, it is crucial to account for this factor when assessing the link between ALI levels and CVD mortality in hypertensive patients (Li et al., 2020). Therefore, we adjusted for BMI and utilized RCS to examine the possible non-linear relationship between ALI and hypertension.

After controlling for BMI, we found that higher levels of ALI remained associated with a lower risk of CVD mortality in hypertensive patients. While elevated ALI levels may correspond to higher BMI levels, our RCS analysis did not reveal any U-shaped relationship. Although the regression analysis indicated that an elevated BMI was linked to a higher risk of CVD mortality when treated as a continuous variable, this association was not significant ($p = 0.091$). On the other hand, our findings revealed that hypertensive patients with a BMI ranging from 24.9 to 35.5 kg/m² had a lower risk of CVD mortality compared to those with a BMI ≤ 24.9 kg/m². Consequently, the results of the regression analysis of BMI as a continuous variable may have been influenced by the severely obese population (BMI >35.5 kg/m²), leading to inconsistent findings. Similar results have been reported in previous studies. For instance, a retrospective study showed that low BMI was linked to an increased 3-year risk of all-cause mortality in hypertensive patients, while obesity was related to a reduced risk of all-cause mortality (Kim et al., 2022). In Zhu et al. (2022)'s study, being underweight was associated with higher mortality in people with high blood pressure, while being overweight was associated with lower mortality. In addition, the study of Zhou et al. (2021) also confirmed that low BMI was an independent risk factor for death in patients with hypertension, while high BMI was not. Obese individuals may have greater metabolic reserves to cope with inflammation and metabolic demands (Doehner et al., 2010). In addition, systemic vascular resistance and plasma renin activity were lower in hypertensive patients with higher BMI compared with those with lower BMI, which can improve the prognosis of hypertensive patients (Lavie et al., 2007). Therefore, although high BMI is a recognized risk factor for CVD death, ALI can still be used as a biomarker for prognosis in hypertensive patients with BMI ≤ 35.5 kg/m².

In the supplementary analysis, it was found that increased levels of ALI were associated with a decreased risk of CVD mortality only in the subgroup with ALI ≤ 60 , which is in line with the RCS findings. This could be attributed to the fact that high ALI may be linked to high BMI, as previously mentioned. The accuracy of ALI may be significantly impacted when BMI >35.5 kg/m², indicating that ALI might not be suitable for individuals with severe obesity.

Previous studies have suggested that low ALI is associated with a higher risk of death and may require early intervention (Jafri et al., 2013; Maeda et al., 2020). Increasing the intake of nutraceuticals and fruits has been shown to be associated with lower levels of inflammation (Scicchitano et al., 2014; Maaliki et al., 2019; Zuraini et al., 2021). As this was a retrospective cohort study, we were unable to confirm whether the use of nutritional supplements and increased fruit intake could improve the risk of CVD death in hypertensive patients. Therefore, these are only hypotheses for the treatment and management of hypertension, and more clinical trials are needed to confirm their effectiveness.

This cohort study, which included 20,517 individuals, has a large sample size that lends reliability to our findings. Our results indicate that hypertensive patients with high ALI levels have a lower risk of CVD death. As a simple and easily calculated index, ALI may provide a more comprehensive assessment of the risk of CVD death in hypertensive patients than a single inflammatory parameter. Early intervention in hypertensive patients with low ALI levels may potentially have a positive effect in reducing the

risk of CVD death. However, further experimental studies are needed to confirm this hypothesis.

Limitations

There are some limitations in our study. First, the diagnosis of hypertension and comorbidities was mostly based on self-reported information, which may have introduced recall bias. Second, the use of a single blood draw may not have provided a complete picture of a patient's physical state, which could change over long-term follow-up. Finally, our study design as a cohort study means that the results should be interpreted as correlational rather than causal, given the possibility of unmeasured confounding factors (Reddy et al., 2018). Therefore, further clinical trials are necessary to confirm our findings.

Conclusion

ALI was an effective indicator of systemic inflammation in hypertensive patients. High levels of ALI were associated with a reduced risk of CVD death in hypertensive patients.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

LC designed the research and is the guarantor of this work. LC had full access to all the data in the study and takes responsibility for

the integrity of the data and the accuracy of the data analysis. JT performed the analyses and wrote the first draft of the paper. BW, JX, JD, SL, JL, YY, PY, JZ, KC, SD, and LC revised the manuscript. All authors read and approved the final manuscript and its submission.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphys.2023.1074672/full#supplementary-material>

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The dose-response effect of aerobic exercise on inflammation in colon cancer survivors

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Background: Physical activity after surgical resection for colon cancer is associated with significantly longer disease-free survival. Inflammation is hypothesized to mediate the association between physical activity and disease-free survival in colon cancer.

Methods: In this exploratory analysis of a randomized dose-response trial, 39 colon cancer survivors who completed standard therapy were stratified by cancer stage and randomized in a 1:1:1 ratio to one of three treatment groups for 24 weeks of usual-care control, 150 min/wk of moderate-intensity aerobic exercise (low-dose), or 300 min/wk of moderate-intensity aerobic exercise (high-dose). Inflammation outcomes included high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL6), and soluble tumor necrosis factor- α receptor 2 (sTNF α R2). Mixed models for repeated measures were used to test the hypothesis that exercise was associated with dose-response reductions in inflammation; exploratory analyses examined treatment effects by cancer stage.

Results: In the overall population, aerobic exercise was not associated with dose-response reductions in hs-CRP, IL6, or sTNF α R2. Cancer stage modified the association between randomized group and hs-CRP ($P=0.022$) and IL6 ($P<0.001$) but not sTNF α R2 ($P=0.39$). In stage I-II disease, compared to control, exercise was not associated with inflammation outcomes. In stage III disease, compared to control, low-dose exercise reduced hs-CRP: -35.4% (95% CI: $-70.1, -0.7$) and IL6: -29.6% (95% CI: $-58.4, -0.8$) but not sTNF α R2: 2.7% (95% CI: sTNF α R2: $-15.7, 21.1$); high-dose exercise was not associated with inflammation outcomes in stage III disease.

Conclusion: This exploratory analysis offers preliminary data to support the hypothesis that inflammation may mediate the association between physical activity and disease-free survival in colon cancer.

Clinical trial registration: clinicaltrials.gov, identifier NCT02250053.

KEYWORDS

biomarkers, C-reactive protein, dose-response, interleukins, tumor necrosis factors

1 Introduction

Physical activity after surgical resection for colon cancer is associated with a significantly longer disease-free survival (1, 2), by reducing the risk of disease recurrence in a subset of patients (3). The association between physical activity and disease-free survival is independent of known prognostic factors and occurs in a dose-response fashion, such that larger volumes of physical activity are associated with a higher probability of remaining alive and cancer-free (4). The biological mechanisms by which physical activity is associated with improved disease-free survival remain incompletely understood, but inflammation is postulated as a key mediator (5).

Inflammation activates the JAK-STAT and NF- κ B signaling pathways to promote cancer cell proliferation, migration, and invasion (6). Inflammation that persists after recovery from colonic tumor resection is independently associated with shorter disease-free survival (7–9). In animal models, experimental manipulation of inflammatory pathways regulates the growth and progression of colonic tumors (10). However, data from clinical trials in colon cancer survivors are limited (11), and the effects of different exercise doses on inflammation outcomes are unknown.

We conducted an exploratory analysis to examine the effects of distinct doses of aerobic exercise using data from a trial that randomized colon cancer survivors to one of three groups for 24 weeks: usual-care control, 150 min/wk of moderate-intensity aerobic exercise (low-dose), or 300 min/wk of moderate-intensity aerobic exercise (high-dose) (12). We hypothesized that exercise would reduce inflammation in a dose-response fashion. Inflammation may correlate with colon cancer disease stage (13). Therefore, we examined if subjects with higher-stage colon cancer derive a larger anti-inflammatory benefit from exercise.

2 Methods

2.1 Study design

This study was a 24-week, phase II, single-center, randomized, dose-response trial. The study followed Good Clinical Practice and the ethical principles in the Declaration of Helsinki. The Institutional Review Board approved the trial protocol and informed consent document. All subjects provided informed consent and approval from their physician before completing any study activities. The study was registered on clinicaltrials.gov as NCT02250053, and detailed study methods are published (12). The prespecified primary and secondary outcomes are published (14–18). The inflammation outcomes reported here were not prespecified and were conducted for exploratory purposes to inform the design of future studies.

2.2 Subjects

Subjects were eligible if they were diagnosed with histologically-proven stage I–III colon cancer, underwent surgical tumor resection, completed postoperative chemotherapy within 36 months of

entering the study (if applicable), self-reported <150 min/wk of moderate- to vigorous-intensity physical activity (19), were age \geq 18 years, provided written physician approval, had no additional surgery planned within the 24 week intervention period, and could walk unassisted for six minutes.

2.3 Randomization and blinding

Subjects were stratified by cancer stage (I vs. II vs. III) and randomized to one of three groups: usual-care control, 150 min/wk of moderate-intensity aerobic exercise (low-dose), or 300 min/wk of moderate-intensity aerobic exercise (high-dose). Subjects were not blinded to treatment assignment. Outcome measures were obtained by assessors blinded to treatment assignment.

2.4 Intervention

Subjects randomized to the low-dose or high-dose exercise groups utilized a study-provided in-home treadmill and heart rate monitor. The exercise intensity was prescribed at 50–70% of the age-predicted maximum heart rate. The low-dose and high-dose target exercise volume was 150 and 300 min/wk, respectively. Subjects were encouraged to individualize their frequency (days per week), fractionation (sessions per day), and duration (minutes per session) of exercise according to a schedule that promoted a high level of adherence to the prescribed exercise volume (17). Subjects randomized to the usual-care control group maintained their pre-study physical activity levels.

2.5 Measurements

Demographic characteristics, including age, sex, and race, were self-reported. Cancer stage was obtained from the cancer registry, pathology reports, and physician records. At baseline and week 24, subjects underwent a fasting blood draw. Blood draws were performed after a minimum eight-hour fast and abstinence from alcohol consumption for 24 hours. A total of 30 mL of plasma was centrifuged, aliquoted, and stored at -80°C following standardized procedures. Circulating tumor cells were measured as previously described (15).

2.6 Inflammatory outcomes

Inflammation measures included high-sensitivity C-reactive protein (hs-CRP), interleukin 6 (IL6), and soluble tumor necrosis factor- α receptor 2 (sTNF α R2). These inflammatory measures are associated with disease-free survival in colon cancer survivors (20–22). hs-CRP was measured as a marker of overall systemic inflammation (23). IL6 was measured as an activator of the JAK-STAT pathway (24). sTNF α R2 was measured as an activator of the NF- κ B pathway (25). sTNF α R2 is a surrogate marker for TNF α that is more stable in plasma and less sensitive to diurnal variation (26).

hs-CRP was quantified using an immunoturbidimetric assay (Roche Diagnostics). IL6 and sTNF α R2 were quantified using ultrasensitive sandwich enzyme immunoassays (R&D Systems). Baseline and week 24 samples were assayed simultaneously and in duplicate at the end of the study. Blinded quality-control samples were interspersed among cases. The median [interquartile range] time from biospecimen collection to laboratory analysis was 3.7 years [3.4, 3.8], and all samples were never previously thawed (27). The coefficients of variation for all samples were $\leq 8\%$ (11).

2.7 Statistical analyses

Descriptive statistics for baseline variables include counts and proportions for categorical variables and means and standard deviations for continuous variables. Dependent variables were log-transformed in the inferential analysis to improve the distributional normality (28). The change was evaluated from baseline to follow-up in the three groups using mixed models for repeated measures. This modeling technique includes all data and accounts for the correlation between measures. Treatment effects were calculated as the treatment effect ratio, which quantifies the percent change in geometric means from baseline to week 24 (e.g., a treatment effect ratio of 0.75 indicates a 25% reduction), with 95% confidence intervals (CI). The regression models included the baseline value of the dependent variable and cancer stage (randomization stratification factor) as covariates to improve the precision of effect size estimation (29). Group-by-time interaction terms were included as fixed effects in the regression model. A test of trends with linear and nonlinear (quadratic) contrasts evaluated the presence of a dose-response relationship across randomized groups. Effect modification by cancer stage was evaluated by including a three-way interaction among cancer stage,

randomized group, and time in the mixed models for repeated measures.

At randomization, cancer stage was a three-level variable (I vs. II vs. III). However, for this analysis, subjects with stage I and II disease were combined ($n=19$) to provide a numeric balance to the number of subjects with stage III disease ($n=20$). The threshold for statistical significance for interactions was prespecified at $P<0.10$, because of known limitations in statistical power (30). Model fit was assessed using graphical and numeric techniques. Stata 17.0 (College Station, TX, USA) was used for all statistical analyses, and GraphPad Prism 9.4 (Boston, MA, USA) for data visualization.

3 Results

Subjects were recruited and randomized between January 2015 and August 2015, with data collection ending in February 2016. The study completion rate was 97% (one subject was lost to follow-up; [Supplementary Figure 1](#)). At baseline, the age ranged from 35 to 81 years, and subjects were most often female (62%), of white race (79%), with stage III disease (51%), and treated with chemotherapy (72%; [Table 1](#)).

At baseline, the mean (SD) hs-CRP was 2.53 (2.11) mg/L, IL6 was 2.07 (1.63) pg/mL, and sTNF α R2 was 2524 (840) pg/mL, indicating low to moderate inflammation. From baseline to week 24, the low-dose and high-dose groups completed an average of 141 min/wk (93% adherence) and 247 min/wk (89% adherence) of exercise, respectively. Exercise adherence ranged from 17–100% and 21–100% in the low-dose and high-dose groups, respectively. The low-dose and high-dose exercise groups averaged 3.5 and 4.3 days of exercise each week and 41.6 and 59.1 minutes per session, respectively. Detailed adherence trajectories have been reported (17).

TABLE 1 Baseline subject characteristics overall and by randomized group.

Characteristic	Overall (n=39)	Control (n=13)	Low-Dose (n=14)	High-Dose (n=12)
Age, years	56.5 \pm 10.0	57.9 \pm 9.7	58.2 \pm 9.8	53.1 \pm 10.5
Sex, %				
Male	15 (38%)	4 (31%)	7 (50%)	4 (33%)
Female	24 (62%)	9 (69%)	7 (50%)	8 (67%)
Race, %				
White	31 (79%)	8 (62%)	12 (86%)	11 (92%)
Nonwhite	8 (21%)	5 (38%)	2 (14%)	1 (8%)
Cancer Stage, %				
I/II	19 (49%)	6 (46%)	7 (50%)	6 (50%)
III	20 (51%)	7 (54%)	7 (50%)	6 (50%)
Chemotherapy, %				
Yes	28 (72%)	10 (77%)	10 (71%)	8 (67%)
No	11 (28%)	3 (23%)	4 (29%)	4 (33%)

Data are mean \pm standard deviation or n (%).

In the overall intention-to-treat population, randomization to higher doses of aerobic exercise was not associated with dose-response reductions in hs-CRP (linear $P=0.74$; nonlinear $P=0.41$), IL6 (linear $P=0.11$; nonlinear $P=0.77$), or sTNF α R2 [(linear $P=0.66$; nonlinear $P=0.75$); Table 2].

At study enrollment, subjects with stage I or II colon cancer had completed cancer-directed treatments a mean of 12.0 (5.6) months previously, and subjects with stage III colon cancer completed cancer-directed treatments a mean of 9.0 (6.1) months previously [Δ : -2.9 months (95% CI: -5.6, -0.3)]. Subjects with stage I and II colon cancer did not have different concentrations of hs-CRP ($P=0.26$), IL6 ($P=0.74$), or sTNF α R2 ($P=0.44$). Cancer stage modified the association between randomized group and hs-CRP ($P_{\text{interaction}}=0.022$) and IL6 ($P_{\text{interaction}}<0.001$) but not sTNF α R2 ($P_{\text{interaction}}=0.39$). Exercise adherence did not differ between subjects with stage I or II versus stage III colon cancer ($P=0.17$).

Compared to control, randomization to low-dose or high-dose aerobic exercise was not associated with inflammation outcomes in subjects with stage I or II colon cancer (Table 3). Conversely, compared to control, randomization to low-dose aerobic exercise statistically significantly reduced hs-CRP: -35.4% (95% CI: -70.1, -0.7) and IL6: -29.6% (95% CI: -58.4, -0.8), but not sTNF α R2: 2.7% (95% CI: -15.7, 21.1) in subjects with stage III cancer, whereas randomization to high-dose aerobic exercise was not associated with a reduction in any inflammation outcome in subjects with stage III colon cancer.

Correlational analyses of inflammation outcomes at baseline and change from baseline to week 24 with previously reported variables are presented for hypothesis generation (Figure 1). Notably, baseline hs-CRP correlated with circulating tumor cells ($r=0.43$; 95% CI: 0.07, 0.68), and the change from baseline to week 24 in sTNF α R2 correlated with the change in circulating tumor cells ($r=-0.44$; 95% CI: -0.72, -0.04).

4 Discussion

In this exploratory analysis of insufficiently physically active colon cancer survivors with low to moderate inflammation at

baseline, randomization to 150 min/wk of moderate-intensity aerobic exercise for 24 weeks reduced concentrations of hs-CRP and IL6 in those with stage III disease. Randomization to 300 min/wk of moderate-intensity aerobic exercise was not associated with any inflammation-lowering effect, nor was either dose of aerobic exercise assigned to those with stage I or II colon cancer. In correlational analyses, inflammation was associated with circulating tumor cell burden at baseline and during follow-up.

One mechanism by which physical activity is hypothesized to exert anticancer effects is by reducing inflammation within the host microenvironment (5). Our results demonstrate that 24 weeks of 150 min/wk of moderate-intensity aerobic exercise reduce hs-CRP and IL6 by 35.4% and 29.6% among stage III colon cancer survivors. In a prospective cohort study of 1,494 stage III colon cancer survivors, elevated hs-CRP and IL6 were associated with a 65% and 52% higher relative risk of disease recurrence or death, respectively (9). Our findings are consistent with the hypothesis that inflammation is a key mediator of the association between physical activity and disease-free survival in colon cancer survivors. Moreover, our results enhance the clinical relevance of experiments in tumor-bearing preclinical models that conclude inflammatory pathway blockade slows cancer cell growth and delays tumor progression (31, 32).

The results of the current analysis complement a prior trial that was conducted as part of the National Cancer Institutes (NCI) Transdisciplinary Research on Energetics and Cancer (TREC) Consortium (33). This prior trial used a 2x2 factorial design to evaluate the effect of 12 weeks of exercise or metformin on inflammation in 139 breast and colorectal cancer patients (11). Compared with control, randomization to 220 min/wk of moderate-intensity aerobic exercise statistically significantly reduced hs-CRP: -30.2% (95% CI, -50.3, -1.0) and IL6: -30.9% (95% CI, -47.3, -9.5); but did not significantly change sTNF α R2: 1.0% (95% CI, -10.4, 13.9) (11). Our results are compatible regarding the specificity of inflammatory biomarker response (e.g., hs-CRP and IL6 were lowered with exercise but not sTNF α R2) and the magnitude of biomarker response (e.g.,

TABLE 2 Change in inflammation outcomes by randomized group.

Inflammation outcome	Randomized group	Baseline geometric mean (SD)	Geometric mean change (SE)	Intervention main effect, treatment ratio (95% CI)	Percent difference between groups (95% CI)
hs-CRP, mg/L	Control	0.07 (1.26)	0.10 (0.14)	1.00 (Reference)	0.00 (Reference)
	Low-Dose	0.60 (1.23)	-0.01 (0.13)	0.90 (0.56, 1.25)	-9.8 (-44.0, 24.5)
	High-Dose	0.58 (1.10)	0.16 (0.14)	1.07 (0.65, 1.49)	6.8 (-35.2, 48.9)
IL6, pg/mL	Control	0.60 (0.60)	-0.23 (0.13)	1.00 (Reference)	0.00 (Reference)
	Low-Dose	0.41 (0.65)	-0.04 (0.11)	1.21 (0.80, 1.62)	20.7 (-20.3, 61.7)
	High-Dose	0.52 (0.75)	0.06 (0.12)	1.34 (0.87, 1.81)	33.6 (-13.4, 80.7)
sTNF α R2, pg/mL	Control	7.65 (0.29)	-0.06 (0.04)	1.00 (Reference)	0.00 (Reference)
	Low-Dose	7.89 (0.27)	-0.03 (0.04)	1.03 (0.91, 1.15)	3.1 (-9.2, 15.4)
	High-Dose	7.79 (0.38)	-0.03 (0.04)	1.03 (0.90, 1.16)	2.8 (-9.9, 15.5)

SD, standard deviation; LS Mean, least squares mean; SE, standard error; CI, confidence interval. Models are adjusted for cancer stage (randomization stratification factor) and baseline value of the dependent variable.

TABLE 3 Change in inflammation outcomes by cancer stage subgroup and randomized group.

Inflammation outcome	Cancer stage subgroup	Randomized group	Baseline geometric mean (SD)	Geometric mean change (SE)	Intervention main effect, treatment ratio (95% CI)	Percent difference between groups (95% CI)	P cancer stage-by-group-by-time interaction
hs-CRP, mg/L	Stage I-II	Control	1.00 (0.29)	0.11 (0.15)	1.00 (Reference)	0.00 (Reference)	0.022
		Low-Dose	0.35 (1.49)	0.37 (0.11)*	1.30 (0.82, 1.78)	29.6 (−18.4, 77.06)	
		High-Dose	1.14 (0.85)	0.33 (0.12)*	1.24 (0.77, 1.71)	24.0 (−23.3, 71.3)	
	Stage III	Control	−0.60 (1.27)	0.05 (0.19)	1.00 (Reference)	0.00 (Reference)	
		Low-Dose	0.84 (0.96)	−0.38 (0.19)*	0.65 (0.30, 0.99)	−35.4 (−70.1, −0.7)	
		High-Dose	0.01 (1.08)	−0.01 (0.21)	0.95 (0.42, 1.48)	−5.3 (−58.2, 47.6)	
IL6, pg/mL	Stage I-II	Control	0.97 (0.48)	−0.49 (0.15)*	1.00 (Reference)	0.00 (Reference)	<0.001
		Low-Dose	0.29 (0.52)	0.35 (0.11)*	2.33 (1.47, 3.18)	132.6 (47.2, 218.1)	
		High-Dose	0.74 (0.84)	0.14 (0.12)	1.89 (1.17, 2.60)	88.9 (17.3, 160.4)	
	Stage III	Control	0.33 (0.56)	−0.08 (0.15)	1.00 (Reference)	0.00 (Reference)	
		Low-Dose	0.53 (0.78)	−0.43 (0.15)*	0.70 (0.42, 0.99)	−29.6 (−58.4, −0.8)	
		High-Dose	0.30 (0.66)	−0.02 (0.16)	1.06 (0.61, 1.52)	6.4 (−38.9, 51.7)	
sTNFαR2, pg/mL	Stage I-II	Control	7.64 (0.22)	0.02 (0.05)	1.00 (Reference)	0.00 (Reference)	0.39
		Low-Dose	7.85 (0.29)	0.02 (0.04)	1.00 (0.86, 1.14)	0.0 (−13.6, 13.7)	
		High-Dose	7.65 (0.27)	−0.01 (0.05)	0.97 (0.83, 1.10)	−3.3 (−16.9, 10.4)	
	Stage III	Control	7.65 (0.35)	−0.11 (0.06)	1.00 (Reference)	0.00 (Reference)	
		Low-Dose	7.96 (0.27)	−0.08 (0.06)	1.03 (0.84, 1.21)	2.7 (−15.7, 21.1)	
		High-Dose	7.93 (0.43)	−0.05 (0.07)	1.06 (0.86, 1.25)	5.6 (−14.1, 25.3)	

SD, standard deviation; LS Mean, least squares mean; SE, standard error; CI, confidence interval. Models are adjusted for the baseline value of the dependent variable.

*" P<0.05 (within group).

−35.4% vs. 30.2% for hs-CRP and −29.6% vs. −30.9 for IL6). The absence of a statistically significant dose-response effect in the current analysis may indicate that the optimal dose of moderate-intensity aerobic exercise to reduce the studied inflammatory biomarkers in colon cancer survivors is between 150 and 220 min/wk.

The current analysis results complement what is known in healthy subjects without a history of cancer. In the Alberta Physical Activity and Breast Cancer Prevention (ALPHA) Trial, 320 postmenopausal women were randomized to 52 weeks of aerobic exercise or a usual care control group. Compared to control, randomization to 225 min/wk of moderate- to vigorous-intensity aerobic exercise statistically significantly reduced hs-CRP: −13% (95% CI: −21, −4), but did not significantly change IL6: −1% (95% CI: −8, 7) or TNFα: 0% (95% CI: −3, 4) (34). The Breast Cancer and Exercise Trial in Alberta (BETA) randomized 400 postmenopausal women to 52 weeks of 150 min/wk or 300 min/wk of aerobic exercise. Compared to 150 min/wk, randomization to 300 min/wk of moderate- to vigorous-intensity aerobic exercise, did not significantly change hs-CRP, IL6, or TNFα (35). The effects of exercise on inflammation in subjects without cancer has been summarized in a meta-analysis (36). These data in subjects without cancer are comparable to those in cancer survivors, such

that the dose-response curve between exercise volume and change in inflammatory outcomes is not linear.

Our hypothesis that patients with higher-stage colon cancer derive a larger anti-inflammatory benefit from exercise was supported. Although our hypothesis was supported, subjects with stage III disease did not have more inflammation than subjects with stage I-II disease. This contrasts with prior reports that inflammation correlates with colon cancer disease stage (13). Aside from the extent of invasion through the bowel wall (T-stage) and lymph node metastases (N-stage), which are used to determine the American Joint Committee on Cancer (AJCC) overall cancer stage (37), the only baseline factor that differed between stage I-II versus stage III colon cancer survivors was the receipt of chemotherapy. However, chemotherapy per se did not modify the association between randomized groups and inflammatory outcomes. Other factors measured after randomization, such as exercise adherence, did not differ between subjects with stage I-II versus stage III disease. The biological explanation of why cancer stage modifies the association between randomized groups and inflammatory outcomes, therefore, remains uncertain. This observation will be prospectively interrogated in an ongoing randomized trial of exercise in colorectal cancer survivors (e.g., NCT03975491).

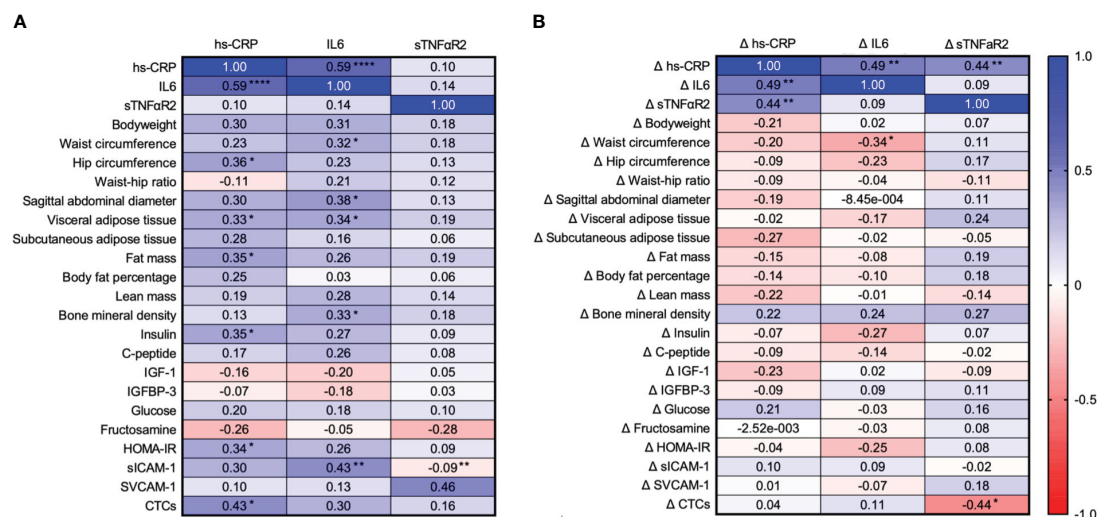


FIGURE 1

Correlational analyses of inflammation outcomes at baseline (A) and change from baseline to week 24 (B) with previously reported variables are presented for hypothesis generation. hs-CRP, high sensitivity C-reactive protein; IL6, interleukin 6; sTNFαR2, soluble tumor necrosis factor alpha receptor 2; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; HOMA-IR, homeostatic model of insulin resistance; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular adhesion molecule 1; CTCs, circulating tumor cells. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

We previously reported that exercise lowers circulating tumor cells using this dataset. Over 24 weeks, statistically significant decreases in circulating tumor cells were observed in the low- and high-dose exercise groups, whereas no significant change was observed in the control group (15). Anthropometric measures, such as visceral fat, and metabolic measures, such as fasting insulin, were biological mediators of the association between exercise and reductions in circulating tumor cells (15). The current analysis suggests inflammation is a potential biological mediator of the association between exercise and reductions in circulating tumor cells. In a cross-sectional study of women with metastatic breast cancer, circulating tumor cells positively correlated with CRP ($r = 0.22$; $P = 0.02$) and IL6 ($r = 0.25$; $P = 0.01$) (38). Changes in circulating tumor cells after surgery and chemotherapy are prognostic of disease-free survival in colorectal cancer survivors (39, 40). Results from an ongoing randomized clinical trial (e.g., NCT03975491) will clarify the interplay of inflammation with circulating tumor cells and circulating tumor DNA to offer unique insight into mechanisms of treatment benefit in colorectal cancer survivors.

There are several limitations to this analysis. The primary limitation is that inflammatory outcomes were not prespecified in the study protocol; consequently, the results of this analysis are hypothesis-generating. The small sample size may have limited our ability to detect small but potentially clinically meaningful effects of exercise on inflammatory outcomes. The sample sizes were very small in the analysis stratified by cancer stage, resulting in uncertainty in the point estimates. The study duration was 24 weeks, which limits our ability to understand the benefits of exercise on inflammatory outcomes acutely and over longer time horizons. Study subjects were not enrolled based on having high inflammation at baseline, which limits our understanding of the

effects of exercise in those with acute or chronic inflammation. We examined two distinct volumes of moderate-intensity aerobic exercise but not the effects of exercise intensity (e.g., light vs. moderate vs. vigorous) or exercise modality (e.g., weightlifting vs. high-intensity interval training) on inflammation outcomes. We examined three inflammation biomarkers associated with disease-free survival in colon cancer survivors (20–22). However, we acknowledge that inflammation can be quantified using many other biomarkers.

There are several strengths to this analysis. The two intervention groups, each prescribed a distinct dose of moderate-intensity aerobic exercise, allowed us to examine how inflammation outcomes change along the exercise dose curve. The aerobic exercise program was flexible, emphasizing home-based exercise, complemented with behavioral coaching from an exercise physiologist. Providing home-based treadmills incentivized study enrollment, as recruitment was completed ahead of schedule, and promoted excellent adherence to the exercise prescription over 24 weeks. Exercise adherence was objectively quantified using heart-rate monitors eliminating bias from self-report. Endpoint data collection, including inflammation assays, was conducted by staff blinded to the study group who adhered to standardized protocols to enhance rigor and reproducibility. Endpoint data collection was excellent (97% follow-up).

In one of the first randomized clinical trials evaluating two doses of moderate-intensity aerobic exercise in colon cancer survivors, this study suggests that 150 min/wk of moderate-intensity aerobic exercise may lower inflammation in select colon cancer survivors. The findings from this exploratory analysis are useful to inform the design of future studies that aim to identify the biological mediators of the relationship between physical activity and disease-free survival in colon cancer survivors.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by University of Pennsylvania School of Medicine. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JB: Writing – original draft, Writing – review & editing. SC: Writing – review & editing. JM: Writing – review & editing. GS: Writing – review & editing. SY: Writing – review & editing.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fonc.2023.1257767/full#supplementary-material>

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Serum vascular adhesion protein-1 is associated with twelve-year risk of incident cancer, cancer mortality, and all-cause mortality: a community-based cohort study

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Background: Vascular adhesion protein-1 (VAP-1), a dual-function glycoprotein, has been reported to play a crucial role in inflammation and tumor progression. We conducted a community-based cohort study to investigate whether serum VAP-1 could be a potential biomarker for predicting incident cancers and mortality.

Method: From 2006 to 2018, we enrolled 889 cancer-free subjects at baseline. Serum VAP-1 levels were measured using a time-resolved immunofluorometric assay. Cancer and vital status of the participants were obtained by linking records with the computerized cancer registry and death certificates in Taiwan.

Results: During a median follow-up of 11.94 years, 69 subjects developed incident cancers and 66 subjects died, including 29 subjects who died from malignancy. Subjects in the highest tertile of serum VAP-1 had a significantly higher risk of cancer incidence ($p=0.0006$), cancer mortality ($p=0.0001$), and all-cause mortality ($p=0.0002$) than subjects in the other tertiles. The adjusted hazard ratios per one standard deviation increase in serum VAP-1 concentrations were 1.28 for cancer incidence (95% CI=1.01–1.62), 1.60 for cancer mortality (95% CI=1.14–2.23), and 1.38 for all-cause mortality (95% CI=1.09–1.75).

The predictive performance of serum VAP-1 was better than that of gender, smoking, body mass index, hypertension, diabetes, and estimated glomerular filtration rate but lower than that of age for cancer incidence, cancer mortality, and all-cause mortality, as evidenced by higher increments in concordance statistics and area under the receiver operating characteristic curve.

Conclusion: Serum VAP-1 levels are associated with a 12-year risk of incident cancer, cancer mortality, and all-cause mortality in a general population.

KEYWORDS

vascular adhesion protein-1, cancer, cancer incidence, cancer mortality, all-cause mortality

Introduction

Based on the Global Cancer Statistics report, the estimated global incidence of cancer for the year 2020 was 19.3 million new cases, while 10.0 million cancer-related deaths occurred worldwide (1). The impact of cancer on global mortality is currently significant, accounting for almost one in six deaths globally. Over the course of the 21st century to date, cancer has surpassed cardiovascular disease as the primary cause of premature death in most countries (2), thereby placing a significant burden on the healthcare system. Implementing preventative measures through the modification of key risk factors presents the most cost-effective long-term strategy for cancer control. Additionally, early detection and timely intervention are crucial factors that can significantly improve the chances of survival for individuals diagnosed with cancer and substantially reduce the financial implications. For this reason, several studies aimed to identify potential biomarkers for the early detection of cancer (3–5).

Inflammation and oxidative stress are important mechanisms involved in many aging-related diseases including cancers, cardiovascular disease, or other diseases associated with disability and mortality (6, 7). Inflammation is recognized as a hallmark feature during the development and progression of cancers. Cytokines, small inflammatory proteins, and infiltrating immune cells derived from tumor and host act in the tumor microenvironment and contribute to the initiation and promotion of carcinogenesis (8). Tumor-derived cytokines and small inflammatory proteins are secreted into systemic circulation and are crucial for the distant metastasis of cancers (8). On the other hand, oxidative stress is recognized as a fundamental process in cancer pathogenesis, contributing to various stages of tumor development and progression, including the transformation of normal cells to tumor cells, tumor proliferation, growth, invasion, angiogenesis, and metastasis (7). Markers of systemic inflammatory and oxidative stress in the circulation could predict cancer progression (9–11). Several studies have reported associations between pre-diagnostic systemic inflammation markers and the risk of developing cancer (12, 13).

Among the various pro-inflammatory proteins, vascular adhesion protein-1 (VAP-1) is notable for its dual functionality.

VAP-1 participates in inflammation and is also a source of oxidative stress. As an endothelial adhesion molecule, it contributes to leukocyte rolling, adhesion, and transmigration into sites of inflammation (14). Additionally, VAP-1 is known to exhibit semicarbazide-sensitive amine oxidase (SSAO) activity, thereby catalyzing the oxidative deamination of primary amines into aldehydes, hydrogen peroxide, and ammonia. This process generates advanced glycation end products (AGEs) and advanced lipoxidation end products (15). AGEs participate in the pathological mechanisms underlying the development of several types of cancer (16). Several investigations have been conducted in recent years to explore the plausible role of VAP-1 in cancers.

VAP-1 has a soluble form that is detectable in circulation, rendering it a biomarker for various diseases. For example, circulating VAP-1 has been shown to correlate with the risk of cardiovascular events (17, 18), the risk of diabetic complications in humans (19), and chronic liver diseases (20). With respect to cancers, serum VAP-1 level could be used to predict the prognosis of colon cancer (21) and gastric cancer (22, 23). In a previous study, we provided evidence for the potential utility of serum VAP-1 levels as a predictive biomarker for incident cancer (24) and mortality in subjects with type 2 diabetes (25). However, it remains unclear whether circulating VAP-1 could prove useful in predicting cancer incidence and mortality in the general population, as opposed to a specific high-risk population. To address this question, the objective of this study was to investigate whether serum VAP-1 can predict the incidence of cancers, cancer mortality, and all-cause mortality in a community-based cohort study.

Materials and methods

Subjects

The study was initiated on 18th December 2007. It was conducted as a prospective cohort study called the Taiwan Lifestyle Study in a community-based setting between 2007 and 2018 (26, 27). We invited residents aged 18 years or older from Yunlin County, Taiwan, to participate in this study, and obtained

written informed consent from each participant. The study underwent review and received approval from the Institutional Review Board of National Taiwan University Hospital (approval number: 200706020R). Participants with a history of cancer at baseline were excluded, since the incidence of cancers was one of the outcome measures. Trained nurses administered a questionnaire to obtain data on demographic characteristics, medical history (including a history of cancer), and health-related lifestyle habits of the participants. We also documented the height and weight of each participant to calculate their body mass index (BMI). Blood pressure was measured using a mercury sphygmomanometer with the arm supported at the heart level after the subject had been sitting calmly for 10 min. Three measurements were taken, and the average of the second and third measurements was used for analysis.

A standard 75-g oral glucose tolerance test (OGTT) was conducted following an 8-h overnight fast. An automatic analyzer (Toshiba TBA 120FR, Toshiba Medical Systems Co., Ltd., Tokyo, Japan) was used to measure plasma glucose and high-sensitivity C-reactive protein (hsCRP) concentrations. Plasma concentrations of hemoglobin A1c (HbA1c) were quantified using automatic analyzers (HLC-723 G7 HPLC systems, Tosoh Corporation, Tokyo, Japan) that were certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized to the Diabetes Control and Complications Trial (DCCT) reference assay. The estimated glomerular filtration rate (eGFR) was calculated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. The serum samples collected for this study were promptly stored at -80°C until the measurement of VAP-1 concentrations was performed. It is noteworthy that serum VAP-1 and its SSAO activity have been previously reported to remain stable for a period of at least 2 years when stored appropriately at -70°C (28). In order to measure serum VAP-1 concentrations, we employed a time-resolved immunofluorometric assay. This involved using a biotin-conjugated monoclonal anti-human VAP-1 antibody (Biotie Therapies Corp., Turku, Finland) as a capturer on a streptavidin-coated microtiter plate. The detection serum VAP-1 bound to the antibody involved the use of a different europium-conjugated anti-human VAP-1 antibody (Biotie Therapies). The resulting time-resolved fluorescence was measured at 615 nm using a fluorometer (Victor² Multilabel Counter, PerkinElmer Finland Oy, Turku, Finland). Serum VAP-1 concentrations were then quantified based on a reference sample of highly purified human serum VAP-1 (Biovian Ltd, Turku, Finland). The standard curves exhibited an R^2 value of 0.997–1.000. Additionally, quality control samples were used to measure the inter-batch coefficients of variation, which ranged from 3.8 to 10.5%.

Outcome measures

The main outcome measures of this study were cancer incidence, cancer mortality, and all-cause mortality. To ascertain the outcomes, we established a linkage between the data from the Taiwan Lifestyle Study and the National Registry of Death and Taiwan Cancer Registry

database using a unique citizen identifying number. Individual participant identification information was deliberately inaccessible during the analysis. The procedure for linking data and subsequent analysis received approval from the Institutional Review Board of National Taiwan University Hospital (approval number: 201412122RINC). The termination date for the follow-up period was 31st December, 2018. Cancer incidence was defined as the frequency of occurrence of new cancers in the cohort per year. Cancer-free participants were those without a diagnosis of cancer until the end of follow-up. Participants who did not experience mortality, as determined by the National Death Registry at the conclusion of the follow-up period, were classified as survivors. In case of mortality, the underlying cause of death was coded according to the International Classification of Diseases, 9th or 10th Revision, Clinical Modification (ICD-9-CM or ICD-10-CM). Death from cancer was coded if ICD-9 = 140–208 or ICD-10 = C00–C96.

Statistical analysis

In this study, normally-distributed continuous variables were reported as means and standard deviations (SD) in metric and S.I. units, while continuous variables with skewed distribution were subjected to logarithmic transformation before analysis and reported as medians (interquartile ranges). Categorical variables were expressed as proportions or percentage of patients in the subgroup. To assess the differences in clinical characteristics between subjects who developed cancer and those who did not and between survivors and non-survivors, Student's *t*-tests and Chi-square tests were performed. Pearson's correlation coefficients were employed to examine the associations between serum VAP-1 concentrations and clinical characteristics as well as plasma biomarkers. The cumulative incidence of cancer, cancer mortality, and all-cause mortality by tertile of serum VAP-1 concentrations was estimated using Kaplan-Meier survival curves and tested by log-rank tests. The associations between outcomes and serum VAP-1 concentrations were assessed using Cox proportional hazard models. We conducted a multivariable analysis and utilized a stepwise procedure to select potential confounding variables for the incidence of cancer. The full model included the variables age, gender, smoking, body mass index (BMI), hypertension, and diabetes mellitus (DM). These variables were chosen based on their potential impact on the development of cancer and their association with serum VAP-1 concentrations. For cancer mortality and all-cause mortality, we included eGFR as a covariate in the models to adjust for potential confounding effects. We utilized the concordance statistics and the area under the receiver-operating characteristic curve (AUC) to assess the predictive ability of the statistical model for cancer incidence, cancer mortality, and all-cause mortality of the participants during the follow-up period. These metrics were expressed in the range of 0.5 (no predictive ability) to 1 (perfect predictive ability). To determine if a given variable could enhance the predictive ability for the outcomes, we calculated the differences in concordance statistics and AUC with and without the variable. We considered a two-tailed *p*-value <0.05 to indicate statistical significance. All statistical analyses were performed using Stata/SE 15.0 for Windows (StataCorp LP, College Station, TX).

Results

This study included 889 subjects enrolled over the period 2006–2010 with a mean age of 62.9 ± 13.9 years. During the median follow-up period of 11.94 years (interquartile range: 10.94–12.97 years), a total of 69 subjects developed incident cancer, and 66 subjects died, including 29 subjects who died from malignancy. Among those with incident cancer, the most prevalent diagnoses were breast cancer ($n=11$), colorectal cancer ($n=9$), hepatobiliary cancer ($n=8$), and lung cancer ($n=8$). Among participants who died from malignancies, the highest proportion was observed for hepatobiliary cancer ($n=8$), followed by lung cancer ($n=5$). Individuals who developed cancer and those who died exhibited higher serum VAP-1 concentrations at baseline (Table 1). In addition, subjects who developed cancers during follow-up were older, had higher systolic blood pressure, HbA1c levels, lower eGFR, and were more likely to have hypertension and/or DM. Subjects who experienced mortality during the follow-up period were characterized by advanced age, male predominance, higher systolic blood pressure (SBP), fasting plasma glucose (FPG), OGTT 2h plasma glucose (OGTT 2hPG), HbA1c, and hsCRP levels, and lower eGFR. Moreover, a greater proportion of these subjects manifested smoking habits, hypertension, and DM.

Table 2 presents the correlation between serum VAP-1 concentrations and the clinical characteristics and plasma biomarkers in the studied subjects. Serum VAP-1 concentration was positively correlated with age, HbA1c, FPG, OGTT 2h PG, and SBP, and negatively correlated with eGFR. No significant associations were found between serum VAP-1 concentrations and BMI, diastolic blood pressure (DBP), or hsCRP levels.

Kaplan-Meier survival curves indicate that the subjects with the highest serum VAP-1 levels had greater incidence of cancer ($p=0.0006$), cancer mortality ($p=0.0001$), and all-cause mortality ($p=0.0002$) compared to other tertiles during the 11.7-year follow-up (Figure 1). The hazard ratios (HRs) of serum VAP-1 for incident cancers, cancer mortality, and all-cause mortality were calculated using Cox proportional hazard models (Table 3). In the univariate analysis, elevated levels of serum VAP-1 were associated with an increased risk of incident cancer, cancer mortality, and all-cause mortality. The adjusted HR per 1 SD increase in serum VAP-1 concentrations in the model developed by forward, backward, and stepwise selection was 1.29 for cancer incidence (95% CI = 1.03–1.08), 1.60 for cancer mortality (95% CI = 1.16–2.19) and 1.39 for all-cause mortality (95% CI = 1.12–1.73). In the full model adjusting for all potential confounders, serum VAP-1 levels could significantly predict incident cancer, cancer mortality, and all-cause mortality. The adjusted HR per 1 SD increase in serum

TABLE 1 Clinical characteristics in subjects with or without cancer incidence and with or without mortality during follow-up.

	No cancer developed	Cancer developed	p	Alive	Dead	p
N (%)	819	69		823 (92.58)	66 (7.42)	
Age (years)	62.03 ± 13.71	73.12 ± 11.64	<0.0001	61.56 ± 13.25	79.67 ± 10.10	<0.0001
Follow up duration (years)	12.12 ± 1.00	5.27 ± 3.15	<0.0001	12.10 ± 1.00	7.06 ± 3.24	<0.0001
Male gender (N, %)	302 (36.87)	31 (44.93)	0.185	292 (35.48)	42 (63.64)	<0.0001
Smoking (N, %)	142(17.36)	16(23.53)	0.134	88 (10.69)	15 (22.73)	0.003
BMI (kg/m ²)	24.34 ± 3.43	24.10 ± 3.76	0.5775	24.31 ± 3.46	24.47 ± 3.51	0.7314
SBP (mmHg)	124 ± 17	128 ± 19	0.0422	124 ± 17	130 ± 18	0.0035
DBP (mmHg)	79 ± 10	79 ± 14	0.7248	79 ± 11	80 ± 10	0.7645
Hypertension (N, %)	239 (29.18)	29 (42.03)	0.026	238 (28.92)	30 (45.45)	0.005
FPG (mg/dL)	92 ± 19	95 ± 20	0.2139	92 ± 17	99 ± 33	0.0029
OGTT 2hr glucose (mg/dL)	125 ± 58	139 ± 69	0.0568	124 ± 54	157 ± 94	<0.0001
HbA1c (%)	5.7 ± 0.8	6.0 ± 1.2	0.0021	5.71 ± 0.74	6.18 ± 1.43	<0.0001
Diabetes mellitus (N, %)	88 (10.74)	13 (18.84)	0.042	81 (9.84)	20 (30.3)	<0.0001
Creatinine (mg/dL)	1 (0.9–1.1)	1.1 (0.9–1.2)	0.0735	1 (0.9–1.1)	1.1(1–1.2)	0.0002
eGFR (mL/min/1.73m ²)	66.96 ± 11.70	60.57 ± 1.31	<0.0001	67.12 ± 11.63	58.22 ± 10.05	<0.0001
hsCRP (mg/dL)	0.09(0.05–0.17)	0.12 (0.05–0.19)	0.3985	0.09 (0.05–0.17)	0.13 (0.05–0.24)	0.0061
Serum VAP-1 (ng/mL)	500.20 ± 142.58	582.71 ± 153.14	<0.0001	498.00 ± 139.19	613.88 ± 171.58	<0.0001

Means ± SD or medians (interquartile ranges) are shown. Plasma triglyceride and hsCRP were logarithmically transformed for statistical analyses.

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; OGTT, oral glucose tolerance tests; HbA1c, glycated hemoglobin; eGFR, estimated glomerular filtration rate by the CKD-EPI equation; hsCRP: high sensitivity C-reactive protein; VAP-1, Vascular adhesion protein-1.

The bold values in the tables signify parameters that exhibit statistically significant differences (p -value <0.05).

TABLE 2 Relationship between serum vascular adhesion protein-1 (VAP-1) concentrations and clinical characteristics.

	R ²	p-value
Age	0.3688	<0.0001
BMI	-0.0493	0.1437
HbA1c	0.2957	<0.0001
Fasting plasma glucose	0.2577	<0.0001
OGTT 2hr glucose	0.3036	<0.0001
SBP	0.1430	<0.0001
DBP	0.0503	0.1346
Log plasma hsCRP	-0.0303	0.3673
eGFR	-0.2615	<0.0001

VAP-1, vascular adhesion protein-1; BMI, body mass index; HbA1c, glycated hemoglobin; OGTT, oral glucose tolerance tests; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate by the CKD-EPI equation; hsCRP: high sensitivity C-reactive protein.

The bold values in the tables signify parameters that exhibit statistically significant differences (p-value <0.05).

VAP-1 concentrations were 1.28 for cancer incidence (95% CI = 1.01–1.62), 1.60 for cancer mortality (95% CI = 1.14–2.23), and 1.38 for all-cause mortality (95% CI = 1.09–1.75).

Table 4 presents the incremental predictive capacity of distinct variables concerning incident cancer, cancer mortality, and all-cause mortality. In the full model that included all predictors, the concordance statistics and AUC were 0.7157 and 0.7276, respectively, for predicting cancer incidence; 0.8595 and 0.8599, respectively, for predicting cancer mortality; 0.8612 and 0.8818, respectively, for predicting all-cause mortality. For the prediction of incident cancer, the increment in concordance statistics and AUC by serum VAP-1 were 0.004 and 0.0057, respectively, which was higher than that of gender, smoking, BMI, hypertension, and DM but lower than that of age. For the prediction of cancer mortality, the respective increments were 0.0179 and 0.0175, which was higher than that of gender, smoking, BMI, hypertension, DM, and eGFR but lower than that of age. For the prediction of all-cause mortality, the respective increments were 0.0085 and 0.0081, which was higher than that of gender, smoking, BMI, hypertension, DM, and eGFR but lower than that of age. These findings suggest that serum VAP-1 can enhance the prediction of cancer incidence, cancer mortality, and all-cause mortality, and that its performance in improving predictions is superior to that of other predictors, with the exception of age.

Discussion

To the best of our knowledge, this study represents the first report that elevated circulating VAP-1 concentrations can independently predict the risk of incident cancers, cancer-related mortality, and all-cause mortality in a general population. The predictive ability of serum VAP-1 for incident cancer was greater than that of gender, smoking, BMI, hypertension, and DM but lower than that of age. The risk of cancer mortality and all-cause

mortality was more reliably predicted by serum VAP-1 than by gender, smoking, BMI, hypertension, DM, and eGFR. Together these results suggest that serum VAP-1 could be a useful biomarker in addition to traditional risk factors to predict incident cancers, cancer mortality, and all-cause mortality.

In the present study, serum VAP-1 emerged as an independent predictor of incident cancer and cancer mortality, even after adjusting for traditional risk factors. The factors of greater age, smoking, and obesity are well established as being significantly associated with an increased risk of cancer development (29). Additionally, individuals with diabetes or hypertension are known to be at higher risk for cancer and cancer mortality (30, 31). Our investigation found a greater increase in concordance statistics and AUC for serum VAP-1 than for gender, smoking, BMI, hypertension, and DM, thereby indicating its superior predictive value in forecasting the occurrence of incident cancer. This finding is supported by previous reports showing the association of VAP-1 with cancer-related events. Individuals diagnosed with hepatocellular cancer exhibited elevated serum VAP-1 levels in comparison to patients with liver cirrhosis alone (20). VAP-1 expression was linked to the progression of tumor invasion and patient survival in breast carcinoma and astrocytoma (32, 33). Among individuals with prostate cancer, serum VAP-1 levels were elevated in those with bone metastases in comparison to those without (34). In addition, previous studies have reported that serum VAP-1 levels were higher in subjects with colorectal cancer compared to healthy volunteers, and that serum VAP-1 levels could serve as an independent prognostic biomarker (21). The collective results of these studies suggest a potential role for VAP-1 in cancer growth and metastasis. However, contrasting results were reported by Toiyama et al., who found that the mean sVAP-1 level was significantly higher in Japanese patients with colorectal cancer than in controls, but the level decreased with disease progression (35). Another study demonstrated that both mean serum VAP-1 levels and tissue VAP-1 protein levels were significantly lower in colorectal cancer patients compared to healthy individuals. However, it is important to note that the sample size of this study was relatively small, consisting of only 31 patients with colorectal cancer and 31 age- and sex-matched controls (36). Comparable results have been reported in individuals with gastric cancer, showing that low sVAP-1 levels were associated with poor prognosis (22, 23). It is worth noting that in our cohort, the number of participants with gastric cancer was limited. Although there may be variations in the relationship between VAP-1 and cancer across different types of cancer, our study provides support for the predictive value of VAP-1 in assessing the risk of cancer incidence and poor prognosis from a general population perspective.

Several potential mechanisms have been proposed to link VAP-1 with cancer progression. VAP-1 has been found to be expressed on tumor vascular endothelium in various types of cancer by immunohistochemical (IHC) staining, such as hepatocellular carcinoma, colorectal cancer, and head and neck cancer, and has also been involved in the recruitment of lymphocytes to cancer vasculature (21, 37, 38). In our previous study, we used IHC analysis to demonstrate a significant upregulation of VAP-1 expression at the invasion front of colorectal cancer compared to its expression in the main tumor. This finding suggests that VAP-1 may play a role

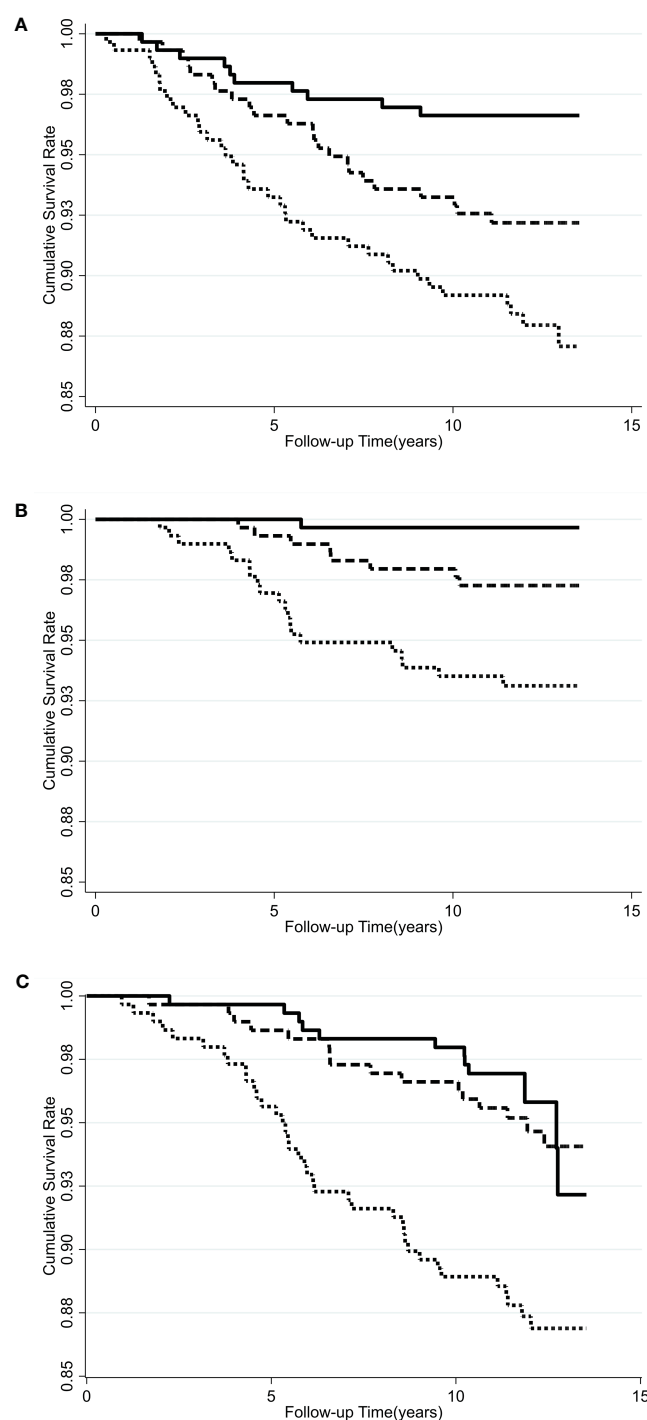


FIGURE 1

Kaplan-Meier curves for the cumulative incidence of subjects who were free of (A) cancers, (B) cancer mortality, and (C) all-cause mortality among different tertiles of serum VAP-1 concentration. Solid line, lowest tertile of plasma VAP-1 concentration; long dash line, middle tertile of serum VAP-1 concentration; dash line, highest tertile of serum VAP-1 concentration. $P = 0.0006$ by log-rank test for incident cancer, 0.0001 for cancer mortality, and 0.0002 for all-cause mortality.

in tumor invasion and metastasis (21). Additionally, VAP-1 has been proposed to promote tumor growth by facilitating the recruitment of Gr-1+CD11b+ myeloid cells into tumors, and increase cancer cell extravasation and angiogenesis in melanoma and lymphoma (39, 40). VAP-1 could also regulate IL-1 β -stimulated M2 macrophage infiltration and induce

lymphangiogenesis and angiogenesis (41). In patients with glioma, VAP-1 expression in tumors was associated with stronger staining of M2 macrophage markers and could be a predictor of a poor prognosis (42). Additionally, VAP-1's SSAO activity could potentially provide another mechanism that links to cancer progression. SSAO is known to catalyze oxidative deamination

TABLE 3 Hazard ratios (HRs) (95% confidence intervals, 95% CI) of serum vascular adhesion protein-1 (VAP-1) concentrations in predicting cancer incidence, cancer mortality, and all-cause mortality in unadjusted and adjusted models.

HRs (95% CIs) per 1 SD increase in serum VAP-1 concentrations	Unadjusted	Adjusted model by stepwise model selection	Adjusted model including all potential confounders
Cancer incidence	1.53* (1.27-1.84)	1.29[‡] (1.03-1.08)	1.28[‡] (1.01-1.62)
Cancer mortality	1.86* (1.46-2.38)	1.60[†] (1.16-2.19)	1.60[†] (1.14-2.23)
All-cause mortality	1.74* (1.46-2.06)	1.39[†] (1.12-1.73)	1.38[†] (1.09-1.75)

One standard deviation (SD) of serum VAP-1 = 144.95 ng/ml.
*p < 0.001, [†]p < 0.01, [‡]p < 0.05.
An adjusted model by stepwise model selection: for cancer incidence, adjusted for age; for cancer mortality, adjusted for age; for all-cause mortality, adjusted for age, smoking, and estimated glomerular filtration rate.
An adjusted model including all potential confounders: for cancer incidence, adjusted for age, gender, smoking, body mass index, hypertension, and diabetes; for cancer mortality, adjusted for age, gender, smoking, body mass index, hypertension, diabetes, and estimated glomerular filtration rate; for all-cause mortality, adjusted for age, gender, smoking, body mass index, hypertension, diabetes, and estimated glomerular filtration rate.
The bold values in the tables signify parameters that exhibit statistically significant differences (p-value <0.05).

reactions that result in the production of hydrogen peroxide, a potent source of oxidative stress, and aldehyde, which is a precursor of AGEs. Both elevated oxidative stress and the interaction between

AGE and its receptor have been associated with the development of cancers (43, 44). Studies have revealed that serum SSAO activity was positively correlated with angiogenic factor VEGF in patients with non-small-cell lung cancer (45). SSAO inhibitors were shown to suppress tumor progression and attenuate neo-angiogenesis of hepatocellular tumors in mice (40). T. Kinoshita et al. have demonstrated in murine colon cancer models that VAP-1 has a role in the generation of an immunosuppressive tumor microenvironment through the H₂O₂-associated Th2/M2 conditions (46). Intraperitoneal administration of the VAP-1 inhibitor U-V296 suppressed tumor growth by enhancing tumor antigen-specific CD8+ T cells. In addition, they also observed a synergistic anti-tumor effect of VAP-1 inhibitors in combination with immune checkpoints inhibitors. In oral squamous cell carcinoma, downregulation of VAP-1 suppressed tumor cell proliferation, migration, and invasion *in vitro* and inhibited tumor proliferation and metastasis *in vivo* through reducing NF-κB/IL-8 signaling and decreasing neutrophil infiltration (47). According to these studies, VAP-1 inhibitors may have a role in treating patients with cancers. It is worth noting that VAP-1 inhibitors have already been developed and are currently under clinical trials for the treatment of diabetic retinopathy and diabetic kidney disease in human (48, 49). Findings from the literature and the present study indicate that exploration of VAP-1 inhibitors in treating cancers are promising and should be performed in the future. In addition, all these findings provide potential mechanisms supporting the association between serum VAP-1 and incident cancers as well as cancer mortality observed in the current study.

TABLE 4 Concordance statistics (C-statistics) and area under the receiver operating characteristic curve (AUC) with and without indicated variables in models predicting cancer incidence, cancer mortality, and all-cause mortality.

	Cancer incidence		Cancer mortality		All-cause mortality	
	C-statistics	AUC	C-statistics	AUC	C-statistics	AUC
Full model	0.7157	0.7276	0.8595	0.8599	0.8612	0.8818
Variable deleted from the model						
Serum VAP-1	0.7117 (0.004)	0.7219 (0.0057)	0.8416 (0.0179)	0.8424 (0.0175)	0.8527 (0.0085)	0.8737 (0.0081)
Age	0.6542 (0.0615)	0.6634 (0.0642)	0.8153 (0.0442)	0.8175 (0.0424)	0.7930 (0.0682)	0.8085 (0.0733)
Gender	0.7168 (-0.0011)	0.7288 (-0.0012)	0.8563 (0.0032)	0.8562 (0.0037)	0.8581 (0.0031)	0.8788 (0.003)
Smoking	0.7168 (-0.0011)	0.7354 (-0.0087)	0.8587 (0.0008)	0.8591 (0.0008)	0.8570 (0.0042)	0.8772 (0.0046)
BMI	0.7202 (-0.0045)	0.7330 (-0.0054)	0.8627 (-0.0032)	0.8623 (-0.0024)	0.8622 (-0.001)	0.8827 (-0.0009)
Hypertension	0.7157 (0)	0.7278 (-0.0002)	0.8571 (0.0024)	0.8584 (0.0015)	0.8615 (-0.0003)	0.8820 (-0.0002)
DM	0.7157 (0)	0.7277 (-0.0001)	0.8586 (0.0009)	0.8588 (0.0011)	0.8614 (-0.0002)	0.8815 (0.0003)
eGFR			0.8613 (-0.0018)	0.8621 (-0.0022)	0.8603 (0.0009)	0.8811 (0.0007)

VAP-1, vascular adhesion protein-1; BMI, body mass index; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate by the CKD-EPI equation. Differences between the full model and the model without the indicated variable are shown in parentheses.

This study has several strengths including the well-characterized clinical parameters, population-based recruitment flow, and accurate records of the incidence of cancers and vital status with a long-term follow-up. Moreover, the utilization of the time-resolved immunofluorometric assay, which possesses high sensitivity, allowed the detection of subtle differences in serum VAP-1 levels. However, some limitations of our study should be considered. First, since the recruited subjects were limited to Han Chinese people, it is unclear whether the present findings can be generalized to other ethnicities. Second, the number of subjects who developed cancers is relatively small. During the 11.94-years follow-up period, a total of 69 subjects developed incident cancer. The annual cancer incidence rate was 0.65%, which is lower than the 1.106% annual cancer incidence rate observed in subjects with diabetes, as presented in our previous reports (24). The predominant cancer types were breast cancer (n=11), colorectal cancer (n=9), hepatobiliary cancer (n=8), and lung cancer (n=8), which is similar to the epidemiological survey in Taiwan (50). Since the numbers for different types of cancer are also limited, we are unable to perform analyses on the relationship between serum VAP-1 and specific cancer types. Future investigations with larger sample sizes will be required to address this issue and provide more comprehensive insights into the association between VAP-1 and various types of cancer.

Conclusion

Our study provides evidence of the association between elevated serum VAP-1 levels and an increased risk of cancer incidence, cancer mortality, and all-cause mortality. These findings indicate that serum VAP-1 might constitute a promising novel biomarker for predicting the probability of incident cancer and mortality in the general population. However, additional investigations are required to clarify the underlying mechanisms and potential clinical applications of our findings, which may ultimately aid in the development of more efficacious screening and treatment approaches.

Data availability statement

The datasets presented in this article are not readily available because the applicability of the data from the National Registry of Death and Taiwan Cancer Registry database is restricted. Requests to access the datasets should be directed to Ministry of Health and Welfare, <https://dep.mohw.gov.tw/DOS/cp-5283-63826-113.html>.

Ethics statement

The studies involving humans were approved by the Institutional Review Board at the National Taiwan University Hospital (202210022RINB). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

SC: Formal Analysis, Methodology, Project administration, Writing – original draft, Conceptualization, Investigation. KF: Data curation, Methodology, Project administration, Writing – review & editing, Formal Analysis, Investigation. IY: Funding acquisition, Methodology, Project administration, Resources, Writing – review & editing, Formal Analysis, Investigation. CY: Investigation, Methodology, Writing – review & editing, Formal Analysis. CL: Data curation, Funding acquisition, Methodology, Project administration, Writing – review & editing, Formal Analysis, Investigation. CH: Methodology, Project administration, Writing – review & editing, Formal Analysis, Investigation. YL: Data curation, Formal Analysis, Writing – review & editing, Investigation, Methodology. HJ: Writing – review & editing, Project administration, Formal Analysis, Data curation, Investigation, Methodology. LH: Data curation, Project administration, Writing – review & editing, Formal Analysis, Investigation, Methodology. ML: Conceptualization, Data curation, Writing – review & editing, Formal Analysis, Investigation, Methodology. SS: Conceptualization, Data curation, Writing – review & editing, Formal Analysis, Investigation, Methodology. HL: Conceptualization, Supervision, Validation, Writing – review & editing, Formal Analysis, Investigation, Methodology. CK: Conceptualization, Methodology, Supervision, Validation, Writing – review & editing, Formal Analysis, Funding acquisition, Investigation, Project administration.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Systemic inflammation among adults with diagnosed and undiagnosed cardiometabolic conditions: a potential missed opportunity for cardiovascular disease prevention

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Context: Systemic inflammation is associated with cardiovascular morbidity and mortality. Since inflammation is not screened in the population, the prevalence, particularly among individuals with undiagnosed cardiometabolic disease, is unclear.

Objective: To assess the prevalence of elevated inflammation using high sensitivity C-reactive protein (hs-CRP) (>0.30 mg/dL) in adults with no cardiometabolic disease, undiagnosed disease and diagnosed disease.

Methods: We conducted a cross-sectional analysis of the 2015–2020 National Health and Nutrition Examination Survey which allows for population estimates of the US population. Adults ≥ 20 years old were included. HsCRP levels >0.30 mg/dL represented inflammation. Individuals were classified into disease defined as having one or more of the following: diagnosed disease--diabetes, hypertension, hyperlipidemia, or obesity by diagnosis; undiagnosed disease (self-report of no doctor diagnosis but positive biomarker); no disease.

Results: 12,946 unweighted individuals representing 315,354,183 adults in the US population were assessed. The proportion of adults with systemic inflammation is 34.63%. The proportion of individuals aged 20 years and older with no disease, undiagnosed disease and diagnosed disease and inflammation was 15.1, 29.1 and 41.8%, respectively. When stratifying by race/ethnicity among individuals with elevated inflammation Non-Hispanic Black people have the highest prevalence (50.35%) in individuals with diagnosed disease followed by Hispanics (46.13%) and Non-Hispanic White people (40.15%) ($p < 0.01$). In logistic regressions adjusted for sociodemographic variables, individuals with undiagnosed cardiometabolic disease have an increased risk of elevated inflammation as measured by CRP (OR 2.38; 95%CI = 1.90–2.99).

Conclusion: In conclusion, a substantial proportion of the adult population, particularly minority and low socioeconomic populations, have elevated inflammation. Systemic inflammation may be a potential focus for disease prevention and disease progression in primary care.

KEYWORDS

National Health and Nutrition Examination Survey (NHANES), cardiovascular disease, inflammation, USA, adults

Introduction

In June 20, 2023, the US Food and Drug Administration approved colchicine as the first anti-inflammatory, atheroprotective cardiovascular treatment (1). The COLCOT trial was a repurposing of colchicine, an anti-inflammatory medication indicated for gout and pericarditis as a secondary prevention for ischemic cardiovascular events among patients who had experienced a myocardial infarction (2). The randomized double-blind placebo controlled trial showed that colchicine led to a significantly lower risk of ischemic cardiovascular events than placebo. In a follow-up randomized double-blind trial among patients with chronic coronary disease, patients on colchicine had significantly lower risk of cardiovascular events than those who received placebo (3). Patients with systemic inflammation, as measured by high sensitivity C-reactive protein (hs-CRP), now have an FDA-approved treatment option demonstrated to reduce the risk of cardiovascular disease by targeting inflammatory pathways.

Systemic inflammation is associated with the development and progression of many chronic conditions like atherosclerosis, diabetes, hypertension, hyperlipidemia, and obesity, as well as morbidity and mortality (4–6). Figure 1 presents a pathway. Evidence has accumulated indicating the significant relevance of low-grade inflammatory processes to cardiovascular disease, cancer and vascular risk factors (4, 7). Further, hs-CRP is a strong independent predictor of future cardiovascular events (6, 8). Cohort studies have shown that elevated CRP is associated with mortality and cardiovascular disease (CVD) events for patients with various CVD locations like coronary artery disease, cerebrovascular disease, peripheral artery disease, and abdominal aortic aneurysm (9).

Inflammation is a modifiable risk factor. Pro-inflammatory diets are associated with increased CVD, cancer, and mortality risk while anti-inflammatory diets may reduce the risk of CVD outcomes (10,

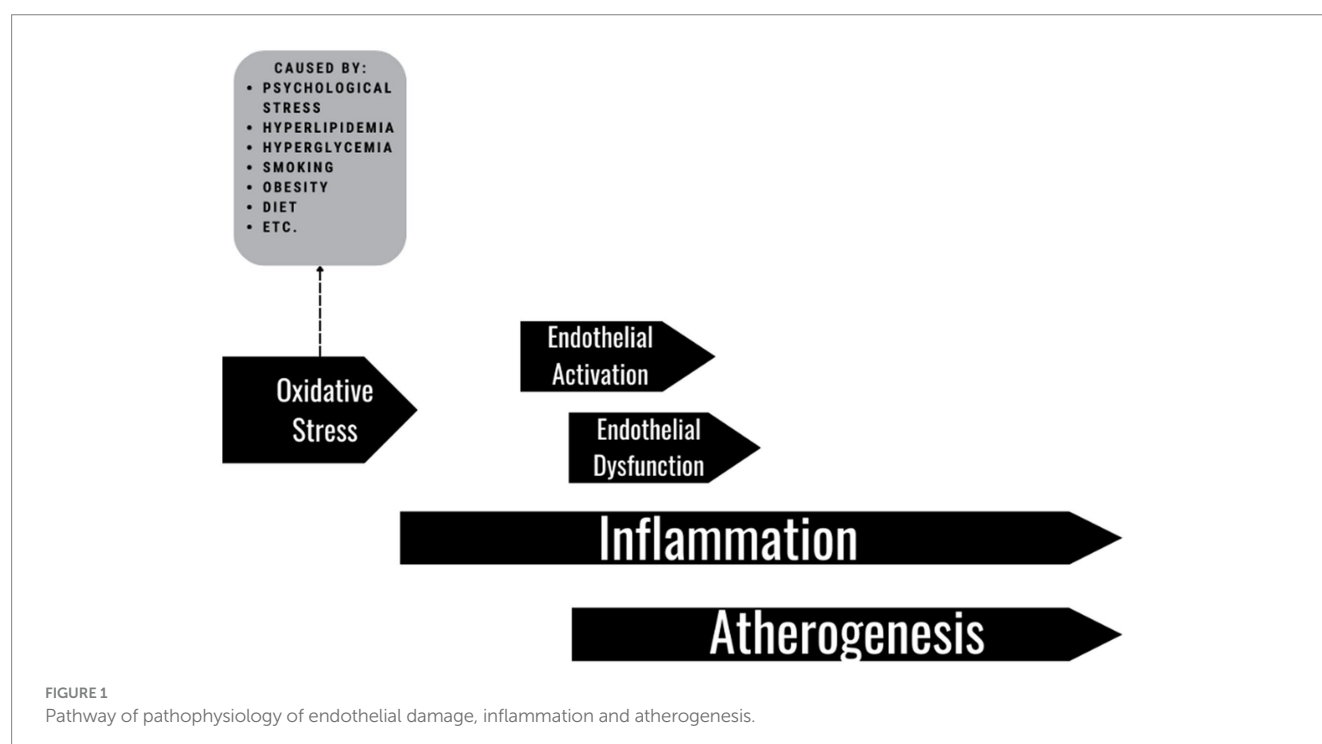
11). Cigarette smoking is also associated with elevated CRP (12). Several major clinical trials have demonstrated that therapy targeted for lowering inflammation will decrease primary and secondary CVD risk (13–15). A variety of medicines have anti-inflammatory effects (13–15). The recent trial of colchicine as an anti-inflammatory medication that spurred the FDA's recent ruling on treatment did show a decreased risk for secondary CVD prevention (3).

Although there is considerable evidence of the link between inflammation and cardiometabolic disease development and progression, and inflammation is a modifiable risk factor, inflammation is not typically measured clinically except in certain autoimmune and infectious diseases. It is unclear whether there is a missed opportunity for prevention of systemic inflammation and concomitant CVD in primary care, particularly for patients with undiagnosed cardiometabolic disease. The goal of this study was to examine the prevalence of systemic inflammation in the population and the possibilities of missed opportunities for CVD prevention.

Methods

In this cross-sectional study, we analyzed the National Health and Nutrition Examination Survey (NHANES) for the years of 2015–2020 prior to Pandemic. The NHANES is a large, nationally representative survey that samples the non-institutionalized population of the United States using a stratified multistage probability sample design. This national, public use, deidentified database is considered “not human subjects” by the Institutional Review Board of the University of Florida.

To account for nationally representative population estimates, the National Center for Health Statistics applies a multilevel weighting system. The survey included a standardized medical examination including blood analysis for examining biomarkers and health-related



interviews. The application of weights and variables accounting for the complex survey design allowed us to provide population estimates. Our study focused on adults aged 20 years-old and older.

Inflammation

HS-CRP was used as an outcome. It was categorized into two levels to indicate inflammation. A threshold of 0.30 mg/dL has demonstrated a significant linkage to the development of cardiometabolic disease. Elevated HS-CRP was defined as >0.3 mg/dL, as recommended by the Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA) (8).

Cardiometabolic conditions

Cardiometabolic conditions were identified by self-reported questionnaire. Individuals were classified into three categories: (1) no cardiometabolic condition, (2) one or more diagnosed chronic disease(s) such as diabetes, hypertension, hyperlipidemia, or obesity determined by a doctor, and (3) at least one undiagnosed chronic disease(s) identified by biomarker(s) and a self-reported survey.

Diabetes

Individuals who had a hemoglobin A1c (HbA1c) level of 6.5% or greater upon examination and who reported never being told by a physician that they had diabetes (excluding gestational) were considered to have undiagnosed diabetes. Individuals who had never been told that they had diabetes and who had an HbA1c level $<6.5\%$ were considered not to have diabetes (16).

Hypertension

Blood pressure was measured using a sphygmomanometer during a physical examination at a mobile examination center. Three measures of blood pressure were obtained, with a fourth attempt for those who had a previous measurement interrupted or incomplete. The first readings of systolic and diastolic blood pressure were analyzed. Undiagnosed hypertension was defined as having a systolic blood pressure of ≥ 140 mm Hg or diastolic blood pressure of ≥ 90 mm Hg for respondents who reported never having been told by a physician that they had high blood pressure or hypertension. This corresponds to the levels recommended by the World Health Organization (17). Individuals who reported never Individuals who reported never being told they had hypertension or high blood pressure who had a systolic blood pressure <140 mm Hg, and a diastolic blood pressure <90 mm Hg were considered not to have hypertension.

Hyperlipidemia

Individuals who had a total cholesterol level of ≥ 200 mg/dL and who were never told by a physician that they had high cholesterol were

considered to have undiagnosed hypercholesterolemia. Individuals who reported never being told they had high cholesterol and who had a total cholesterol level of <200 mg/dL were considered not to have hypercholesterolemia (18).

Obesity

Individuals who had been told by a physician that they were overweight and had a body mass index (BMI) ≥ 30 kg/m² were considered to have been diagnosed with obesity [World Health Organization (19)]. Persons who were never told by a physician that they were overweight and had a BMI consistent with obesity were considered to have undiagnosed obesity. Individuals who were never told by a physician and had BMI <30 were not considered to be obese.

Covariates

Covariates included demographics and socioeconomic status (SES). Demographics included age, sex and race/ethnicity and SES included education and the poverty-income ratio. The poverty-income ratio was included because of the implications of financial and psychological stress on inflammation. Race/ethnicity was categorized into four groups: (1) Non-Hispanic White, (2) Non-Hispanic Black, (3) Hispanics, and (4) Other.

Statistical analyses

Descriptive analyses were conducted including ANOVA for mean differences and chi-square tests for categorical variables. Prevalence of elevated hs-CRP was measured, and ANOVA was used to examine significant differences in prevalence across three groups. Unadjusted logistic regression and adjusted regression model controlling age, sex, race/ethnicity, education, and poverty income ratio were used to determine associations between diagnosis status of cardiometabolic disease and elevated hs-CRP. Analyses were conducted using the SAS survey package in version 9.4.

Results

The total study population was 12,946 representing 315,354,183 individuals. The prevalence of diagnosed cardiometabolic disease(s) and undiagnosed cardiometabolic disease are displayed in Table 1. The prevalence of elevated hs-CRP was highest in diagnosed disease followed by those with undiagnosed diseases and those with no evidence of disease. The proportion of adults with systemic inflammation is 34.63%. The proportion of individuals aged 20 years and older with no disease, undiagnosed disease and diagnosed disease and inflammation was 15.1, 29.1 and 41.8%, respectively. A higher proportion of Non-Hispanic Black people and Hispanics than Non-Hispanic White people exhibited undiagnosed disease.

Among individuals with elevated hs-CRP, Non-Hispanic Black people and Hispanic people had higher proportions than Non-Hispanic White people, particularly among adults with undiagnosed and diagnosed cardiometabolic disease ($p < 0.01$) (Table 2). Education

TABLE 1 Population estimates for demographic characteristics of diagnosed, undiagnosed, and no cardiometabolic disease among adults aged ≥ 20 years, 2015–2020 (Unweighted $N = 12,946$; Weighted $N = 315,354,183$).

Factors	Diagnosed disease	Undiagnosed disease	No evidence of disease	<i>p</i> value
Unweighted sample size	8,640	2086	2,220	–
Weighted population size	203,421,624	52,308,003	59,624,556	–
Weighted prevalence of Elevated hs-CRP (%)	41.77	29.13	15.11	<0.01
Age (years)				<0.01
20–44 years	19.38	47.05	33.57	
45–64 years	73.47	17.05	9.48	
65 and above	86.15	9.87	3.98	
Sex (Male) %	63.28	16.96	19.76	0.17
Race/ethnicity (%)				<0.01
Non-Hispanic White	66.56	15.54	17.90	
Non-Hispanic Black	64.83	15.86	19.31	
Hispanic	58.69	20.51	20.80	
Other	59.99	17.93	22.08	
Education (%)				0.02
High School or Less	64.27	18.27	17.46	
Some College or Graduate	64.62	15.64	19.74	
Poverty Income Ratio (%)				0.01
At/below Poverty Line	59.83	18.76	21.41	
Above Poverty Line	65.79	15.99	18.22	

TABLE 2 Prevalence of elevated hs-CRP by diagnosed, undiagnosed and no evidence of cardiometabolic disease stratified by sex, race/ethnicity, education and poverty-income ratio.

Factors	Diagnosed disease	Undiagnosed disease	No evidence of disease
Sex (%)			
Male	16.63	12.30	5.91
Female	25.14	16.82	9.20
Race/ethnicity (%)			
Non-Hispanic White	40.15	25.31	14.68
Non-Hispanic Black	50.35	37.82	15.06
Hispanic	46.13	37.61	18.88
Other	36.35	26.62	11.70
Education (%)			
High School or Less	45.59	36.16	17.97
Some College or Graduate	39.63	24.46	13.68
Poverty Income Ratio (%)			
At/below Poverty Line	46.19	38.84	15.84
Above Poverty Line	40.66	25.98	14.87

The percentages in the [Table 2](#) represents the row percentage of proportion of individuals with high hs-CRP across diagnosed, undiagnosed and no evidence of cardiometabolic disease further segmented by sex, race/ethnicity, education, and poverty income ratio.

attainment and poverty to income ratio (PIR) showed significant differences in the prevalence of elevated hs-CRP ([Table 2](#), $p < 0.05$). Lower SES populations also showed elevated inflammation. Low education attainment and low PIR accounted for higher prevalence of

hs-CRP. Individuals who were at or below the poverty line or those with lower educational attainment showed significantly higher prevalence of elevated hs-CRP, particularly among those with undiagnosed or diagnosed cardiometabolic disease ($p < 0.05$).

TABLE 3 Unadjusted and adjusted logistic regression model examining the association of elevated hs-CRP and disease diagnosis status.

Disease diagnosis status	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio** (95% CI)
Hs-CRP		
No evidence of disease	1.00	1.00
Undiagnosed	2.31 (1.85–2.88)	2.38 (1.90–2.99)
Diagnosed	4.03 (3.33–4.88)	4.53 (3.65–5.62)

**Controlling for Age, Sex, Race/ethnicity, Education and Poverty income ratio.

Table 3 presents the results of the unadjusted and adjusted odds ratio from the logistic regressions. In unadjusted analyses, individuals with undiagnosed cardiometabolic disease were along with those with diagnosed disease to be significantly more likely to show elevated hs-CRP as compared to those with no evidence of disease. In the analyses adjusted for demographic and SES variables, the odds of having increased hs-CRP were increased by 138% for those with undiagnosed disease and were increased by 353% in those with diagnosed disease as compared to normal individuals.

Discussion

The study's findings revealed a significant association between inflammation (hs-CRP) and diagnosed and diagnosed cardiometabolic disease. To our knowledge, this is the first study identifying the significance of elevated hs-CRP among individuals with undiagnosed diseases. These findings emphasize the importance of clinical investigation of hs-CRP in a primary care context for high-risk populations to potentially treat inflammation as a strategy to prevent cardiovascular events and death (20). This evidence further supports the development of patient-centered services in medical practice, there is a need to update guidelines on cardiometabolic diseases with inflammation as a risk factor for better patient outcomes. Non-Hispanic Black people and Hispanic people and individuals with low SES were shown here to have a higher prevalence of inflammation. These individuals are at high risk for cardiovascular morbidity and mortality. Since inflammation is not typically a focus of screening or CVD prevention, it is essential to pay attention to the healthcare needs of these vulnerable people.

Previous studies demonstrated substantial evidence of the link between elevated hs-CRP (as an inflammatory biomarker) and progression of cardiometabolic disease. Several studies have examined that hs-CRP is an established independent risk factor and can predict cardiovascular disease events and its recurrence (8, 9). In accordance with the present results, previous studies have demonstrated that incidence of major cardiovascular events can be reduced by treatment of inflammation (by lowering hs-CRP levels) in undiagnosed individuals (13, 15). However, inflammation is considered a modifiable risk but is not usually measured clinically except for certain autoimmune and infectious diseases. Therefore, screening high-risk populations for elevated hs-CRP and early initiation of anti-inflammatory treatment would contribute to the reduction of the risk of cardiometabolic diseases.

This study has several strengths. The use of the NHANES dataset, which has a complex sampling methodology to provide nationally

representative population estimates of the US population, many millions of people, is one of the study's key strengths. In addition, this study examined the inflammation prevalence among populations with undetected diseases.

There are also several limitations to this study. One of the limitations includes selection of hs-CRP was the primary inflammatory marker we used to identify disease risk. However, although there are other inflammatory markers of chronic diseases, hs-CRP is a standard method to assess the risk of developing cardiometabolic diseases. A second limitation study is that the "Other" racial/ethnic group includes adults self-identified by multiple racial/ethnic designations. Third, the NHANES does not contain information on active infection or malignancies that could significantly raise inflammatory marker levels. Further, there are a variety of variables that we used that are measures of risk factors for cardiovascular disease (e.g., hypertension, hyperlipidemia) but the NHANES does not contain direct measures of clinical cardiovascular disease (e.g., coronary artery calcium, carotid ultrasound). There are many variables that are collected but as a population-based survey the medical history is limited.

To conclude, there exists a missed opportunity for prevention of systemic inflammation particularly for undiagnosed cardiometabolic disease. Systemic inflammation may be useful to assess as a potential focus for disease prevention and disease progression in primary care. Such a focus could have particular benefits among vulnerable populations who are at increased risk for CVD morbidity and mortality.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: <https://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Questionnaire&Cycle=2017-2020>.

Author contributions

AM: Conceptualization, Data curation, Methodology, Writing – original draft, Writing – review & editing. PS: Conceptualization, Formal analysis, Writing – review & editing. AJ: Formal analysis, Writing – review & editing.

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Inflammation and poverty as individual and combined predictors of 15-year mortality risk in middle aged and older adults in the US

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Background: Chronic systemic inflammation and poverty are both linked to an increased mortality risk. The goal of this study was to determine if there is a synergistic effect of the presence of inflammation and poverty on the 15-year risk of all-cause, heart disease and cancer mortality among US adults.

Methods: We analyzed the nationally representative National Health and Nutrition Examination Survey (NHANES) 1999 to 2002 with linked records to the National Death Index through the date December 31, 2019. Among adults aged 40 and older, 15-year mortality risk associated with inflammation, C-reactive protein (CRP), and poverty was assessed in Cox regressions. All-cause, heart disease and cancer mortality were the outcomes.

Results: Individuals with elevated CRP at 1.0 mg/dL and poverty were at greater risk of 15-year adjusted, all-cause mortality (HR = 2.45; 95% CI 1.64, 3.67) than individuals with low CRP and were above poverty. For individuals with just one at risk characteristic, low inflammation/poverty (HR = 1.58; 95% CI 1.30, 1.93), inflammation/above poverty (HR = 1.59; 95% CI 1.31, 1.93) the mortality risk was essentially the same and substantially lower than the risk for adults with both. Individuals with both elevated inflammation and living in poverty experience a 15-year heart disease mortality risk elevated by 127% and 15-year cancer mortality elevated by 196%.

Discussion: This study extends the past research showing an increased mortality risk for poverty and systemic inflammation to indicate that there is a potential synergistic effect for increased mortality risk when an adult has both increased inflammation and is living in poverty.

KEYWORDS

National Health and Nutrition Examination Survey, mortality, cohort, poverty, inflammation

Introduction

Systemic inflammation is associated with the development and progression of many chronic conditions, cardiovascular (CVD), metabolic, renal and oncologic diseases, as well as morbidity and mortality (1–3). Evidence has accumulated indicating the significant relevance of low-grade inflammatory processes to cardiovascular disease, cancer and vascular risk factors (1, 4). Further, high sensitivity C-reactive protein (hs-CRP) is a strong independent predictor of future cardiovascular events (3, 5). Cohort studies have shown that elevated CRP is associated with mortality and cardiovascular disease (CVD) events for patients with various CVD locations like coronary artery disease, cerebrovascular disease, peripheral artery disease, and abdominal aortic aneurysm (6).

Risk factors such as age, diet, lifestyle, and environmental pollutants impact the biochemical and genetic pathways that lead to states of chronic inflammation (1, 4, 7–9). In patients with known cardiovascular disease (CVD), elevated CRP is associated with an increased risk for future CVD events and mortality (6). Elevated CRP is associated with elevated all-cause mortality risk (10–13). One meta-analysis has linked elevated CRP to both all-cause and CVD mortality risk (14).

An estimated 37.9 million Americans (11.6%) were living in poverty in 2021 (15). Poverty negatively affects the health of individuals (16). Poverty correlates with lower life expectancy and premature mortality risk (17–19). Poverty is also linked to increased inflammation (20–21). However, because poverty and inflammation are correlated and both associated with mortality risk but at the same time independent of each other, it is unclear if they act synergistically for mortality and in particular, heart disease and cancer mortality.

This cohort study will provide US population estimates of 15-year mortality risk for the individual and combined presence of poverty and systemic inflammation among middle age and older adults with baseline assessments of poverty and systemic inflammation.

Methods

This study is an analysis of the publicly available, deidentified National Center for Health Statistics NHANES data linked to the National Death Index by the National Center for Health Statistics. We analyzed the National Health and Nutrition Examination Survey (NHANES) 1999 to 2002 with linked records to the National Death Index (NDI) through the date December 31, 2019. The NHANES uses a stratified multistage probability sample design to be representative of the United States (US) population. Participation in NHANES includes providing information through surveys, physical and physiologic examinations, and laboratory assays. The 1999 to 2002 baseline NHANES sample included 3,478 unweighted participants aged 40 and older. We limited the baseline cohort to individuals assessed in the four-year NHANES data collection period of 1999–2002. We had that group as our cohort so that included individuals would be available to be followed for 15-year mortality risk by the end of the available NDI data. The NHANES is an ongoing survey and later years are available but the later years would not allow for linking to the NDI and still allow a 15 year follow-up. As a population-based cohort with a complex survey design and appropriate weighting the

design provides a population estimate representative of the noninstitutionalized US population.

Study cohort definition

The individuals included in this study cohort were aged 40 and older at baseline. Patients were included if they participated in the NHANES 1999–2002 and had the key variables of inflammation, poverty and associated demographics. There was no blinding in this retrospective cohort and the linkage of the data from the NHANES to the NDI was provided by the National Center for Health Statistics and released as a deidentified, public use database. By using middle aged and older adults at baseline it improves the ability to focus on downstream mortality over the next 15 years.

Inflammation

Inflammation was defined at baseline by means of CRP levels. The NHANES reported CRP levels for all of the participants in our study. We categorized elevated inflammation in two different ways. First, elevated CRP was defined as >0.3 mg/dL, as recommended by the Centers for Disease Control and Prevention and the American Heart Association (5). This level was based on evidence of chronic systemic inflammation and CVD risk. Second, in an additional analysis we defined elevated CRP as >1.0 mg/dL which is consistent with systemic inflammation (22).

Poverty

Poverty was defined according to the poverty index ratio which is a standard measure of total family income divided by the poverty threshold. The poverty threshold accounts for the size of the family and the number of related children in the household under 18. Poverty at baseline simply defines whether the individual was living below the poverty line as a baseline exposure characteristic in 1999 to 2002. We categorized individuals in the sample into two groups: (a) Persons without poverty at baseline (“above poverty”) had a poverty income ratio above 1 indicating that the person was not in poverty at baseline and, (b) Persons with poverty at baseline (“poverty”) had a poverty income ratio at or below 1 indicating that the person was in poverty at baseline.

Mortality

The National Center for Health Statistics (NCHS) has linked data collected from the NHANES with death certificate records from the National Death Index (NDI). The mortality status for each participant was censored at 15 years to create consistency among follow-up lengths between members of the different NHANES cohorts. This study used the public use linked mortality files for the nine cause-specific death categories produced by the NCHS [Public-use Linked Mortality File Readme (cdc.gov)]. The NCHS recoded 113 underlying causes of death into several categories. We examined all-cause mortality, heart disease mortality and cancer mortality.

Analysis

We classified the population into 4 groups based on inflammation and poverty (above poverty/low inflammation; above poverty/elevated inflammation; poverty/low inflammation; and poverty/elevated inflammation). We used sampling weights to calculate prevalence estimates for the civilian noninstitutionalized US population. All analyses were conducted using the survey package in R 4.3.3 to account for the complex NHANES sampling design and make population estimates. Thus, the analysis represented a population of approximately 95 million people.

Using the population estimates, we graphically show the cumulative mortality as the unadjusted relationship by the 4 inflammation/poverty groups. We performed Cox proportional hazards analysis with mortality time for each group, controlling for age, sex, and race/ethnicity. We defined elevated inflammation in one analysis as CRP >0.3 mg/dL and in a second analysis elevated inflammation was defined as >1.0 mg/dL. We used as the outcomes 15-year risk of all-cause mortality, heart disease and cancer mortality.

Results

The characteristics of the sample are shown in Table 1. The individuals who live in poverty account for 11.4% of the population. Figure 1 presents the Kaplan–Meier curves for all four groups using two different CRP cutpoints. Figure 1A has a CRP cut off of 0.3 mg/dL to define high inflammation. Figure 1B shows a CRP cut off of 1.0 mg/dL to define high inflammation. These displays of the relationship between inflammation/poverty and mortality over 15 years are unadjusted for variables like age and race/ethnicity, but they convey the general mortality risk. In particular, when using a CRP cut off of

1.0 mg/dL, the above poverty/low inflammation group has lower mortality than the two intermediate groups (high inflammation/above poverty and low inflammation/high poverty) while the poverty/high inflammation group has the highest mortality over 15 years.

The adjusted Cox proportional hazard analysis for mortality risk featured in Table 2 confirmed the trends seen in the unadjusted Kaplan–Meier curves. The results in the analysis with inflammation defined as CRP 0.3 mg/dL suggests that individuals with high CRP levels are at basically equal increased mortality risk whether they are living in poverty or are above the poverty level. However, the analysis which defines inflammation as CRP at 1.0 mg/dL shows that there is a synergistic effect on mortality risk when a person has both elevated inflammation and is living in poverty.

In addition to the all-cause mortality analyses, we also conducted two another analyses for death from heart disease and cancer, respectively. Table 3 shows that when inflammation is defined as CRP 1.0 mg/dL, individuals with both elevated inflammation and living in poverty experience a 15-year heart disease mortality risk elevated by 127% and 15-year cancer mortality elevated by 196%.

Discussion

The results of this study reinforce the findings of previous research that both elevated systemic inflammation and poverty are risk factors for mortality. This study extends the past research to indicate that there is a potential synergistic effect for increased mortality risk when an adult has both increased inflammation and is living in poverty. This effect is specifically observed when inflammation is defined as CRP >1.0 mg/dL. However, inflammation is considered a modifiable risk but is not usually measured clinically except for certain autoimmune and infectious diseases. Therefore, screening high-risk populations for

TABLE 1 Population estimates for demographic characteristics of all four groups among adults aged ≥ 40 years with CRP at 0.3 mg/dL, 1999–2002 (Unweighted $N = 4,849$; Weighted $N = 94,821,664$).

Characteristics	Low inflammation/ above poverty	High inflammation/ above poverty	Low inflammation/ poverty	High inflammation/ poverty	Value of p
Unweighted sample size	2,287	1766	389	407	
Weighted population size	50,031,662	33,893,028	5,449,080	5,447,894	
Sex (%)					
Male	56	39	47	35	
Female	44	61	53	65	
Age (%)					<0.001
40–49 years	44	32	42	36	
50–59 years	27	29	20	28	
60–69 years	16	21	17	15	
70–79 years	11	13	16	18	
80 years & above	3.4	4.2	4.5	3.4	
Race/ethnicity (%)					<0.001
Non-Hispanic White	82	79	55	50	
Non-Hispanic Black	6.5	9.9	13	19	
Mexican American	8.2	9.0	24.2	21.6	
Other Race	3.3	1.9	6.8	10.0	

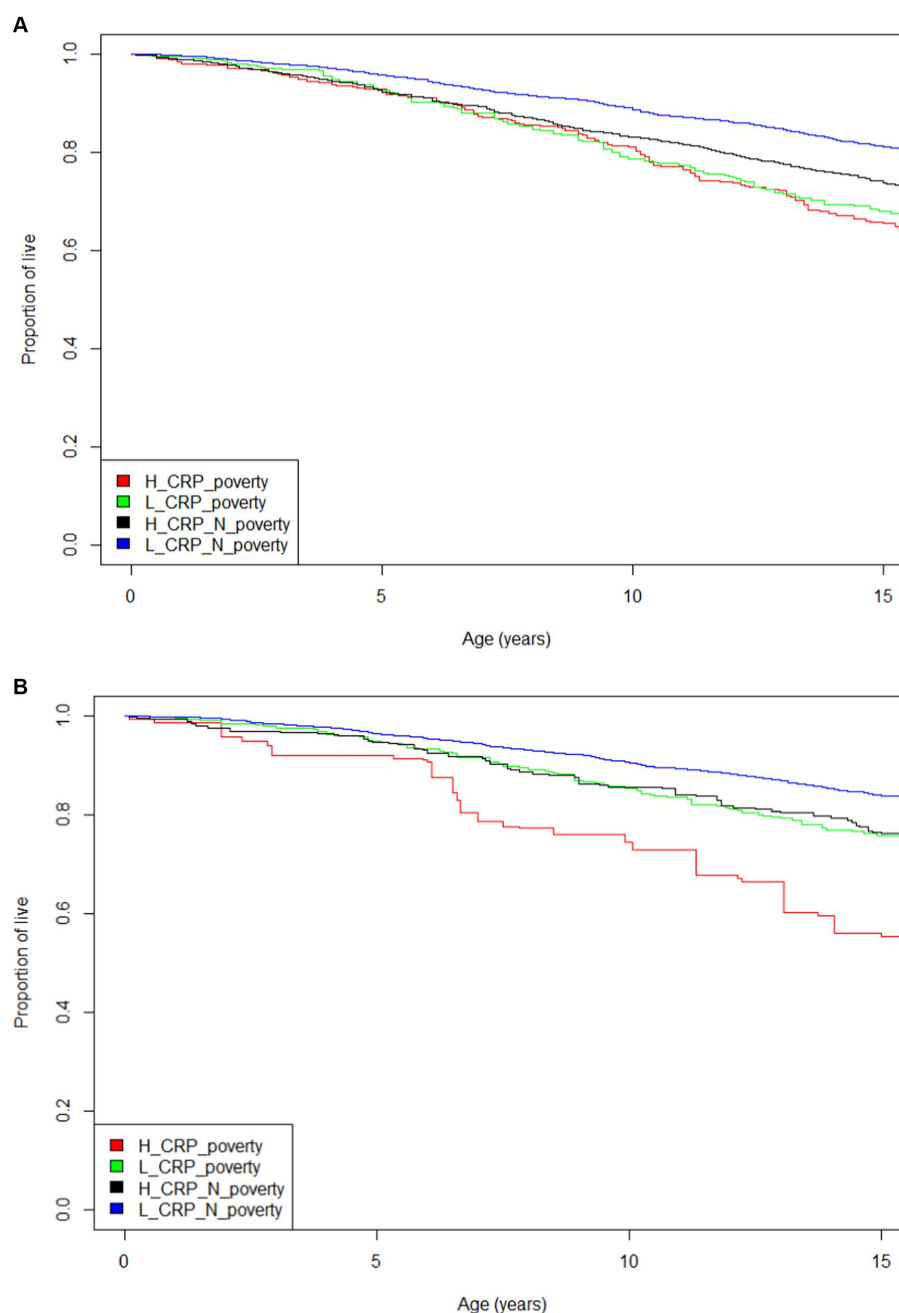


FIGURE 1

(A) Mortality Kaplan–Meier curve for four inflammation and poverty groups and 15 year mortality with CRP = 0.3 mg/dL (log rank test $p < 0.001$).

(B) Mortality Kaplan–Meier curve for four inflammation and poverty groups and 15 year mortality with CRP = 1.0 mg/dL (log rank test $p < 0.001$).

elevated hs-CRP and early initiation of anti-inflammatory treatment, potentially diet or even medications would contribute to the reduction of the risk of future disease and as is shown here, mortality.

This study is clinically relevant because both inflammation and poverty are modifiable risk factors. It emphasizes that focusing only on one of the variables, poverty or inflammation, will still not reduce the mortality risk to that of individuals living above the poverty level with no systemic inflammation. Inflammation and corresponding mortality risk could potentially be reduced by anti-inflammatory diets or potentially anti-inflammatory medications (23–26).

Impoverished social conditions and the underlying impetuses forming them are the basis for preventable disparities in various health outcomes (27). The diet among people in poverty as well as their stress levels contribute to a higher risk of systemic inflammation. It may be useful to target individuals living in poverty for screening for systemic inflammation. It is therefore imperative to also have a better clinical understanding of poverty's relationship with chronic disease morbidity and mortality to guide future screening and outcome studies on social determinants of health.

In June 20, 2023, the US Food and Drug Administration approved colchicine as the first anti-inflammatory, atheroprotective

TABLE 2 Cox regression model for all cause-mortality risk among the 4 groups at CRP 0.3 and 1.0 mg/dL.

All-cause mortality	Hazard ratio at CRP 0.3 mg/dL		Hazard ratio at CRP 1.0 mg/dL	
	Unadjusted (95%CI)	Adjusted (95%CI)**	Unadjusted (95%CI)	Adjusted (95%CI)**
Low inflammation/above poverty line*	--	--	--	--
Low inflammation/poverty	1.47 (1.28–1.68)	1.35 (1.17–1.55)	1.55 (1.30–1.84)	1.58 (1.30–1.93)
Hi inflammation/above poverty	1.86 (1.33–2.60)	1.85 (1.53–2.24)	1.55 (1.22–1.98)	1.59 (1.31–1.93)
Hi inflammation/poverty	2.00 (1.60–2.50)	1.91 (1.43–2.54)	2.53 (1.68–3.81)	2.45 (1.64–3.67)

*Reference category; **Adjusted HR for age, sex, race/ethnicity.

TABLE 3 Cox regression model for heart disease and cancer mortality among the 4 groups at CRP 0.3 and 1.0 mg/dL.

	Hazard ratio at CRP 0.3 mg/dL		Hazard ratio at CRP 1.0 mg/dL	
	Unadjusted (95%CI)	Adjusted (95%CI)**	Unadjusted (95%CI)	Adjusted (95%CI)**
Heart disease				
Low inflammation/above poverty line*	--	--	--	--
Low inflammation/poverty	1.38 (1.06–1.78)	1.27 (0.94–1.71)	1.51 (1.08–2.11)	1.57 (1.10–2.23)
High inflammation/above poverty	1.67 (1.06–2.61)	1.82 (1.15–2.88)	1.54 (1.17–2.04)	1.71 (1.26–2.31)
High inflammation/poverty	1.95 (1.33–2.85)	1.97 (1.25–3.10)	2.20 (1.27–3.82)	2.27 (1.23–4.19)
Cancer				
Low inflammation/above poverty line*	--	--	--	---
Low inflammation/poverty	1.28 (0.91–1.81)	1.25 (0.87–1.81)	1.36 (0.94–1.97)	1.47 (1.00–2.16)
Hi inflammation/above poverty	1.60 (0.97–2.65)	1.78 (1.14–2.78)	1.13 (0.75–1.69)	1.29 (0.87–1.92)
Hi inflammation/poverty	1.37 (0.75–2.53)	1.53 (0.79–2.98)	2.64 (1.40–5.00)	2.96 (1.56–5.59)

*Reference category; **Adjusted HR for age, sex, race/ethnicity.

cardiovascular treatment (28). Specifically, patients with systemic inflammation, as measured by hs-CRP, now have an FDA-approved treatment option demonstrated to reduce the risk of cardiovascular disease by targeting inflammatory pathways.

This study has several strengths and limitations. In terms of strengths, this is a population-based cohort that allows us to make estimates for the non-institutionalized adult population of the US. Second, the inflammation measures, CRP, are standard measures and were collected in a standardized way for everyone. They were not based on a patient being symptomatic, which would likely be the case in an analysis from an electronic health record.

There are some limitations to the study. First, as with any cohort study, there is a general assumption in observational studies that the baseline exposure variable (e.g., inflammation, poverty) has a certain degree of constancy or has had such a physiological insult to the person that it carries over to the downstream mortality risk. Several economic crises have occurred with a relevant impact on the United States population. The key variables in the study may have been affected by the so-called “cohort effect.” The findings may have been affected by that and may not be reliably representative of the current US population. In this case, since systemic inflammation is not

universally recommended for screening at either CRP 0.03 mg/dL or CRP 1.0 mg/dL, it is unlikely that there would be any interventions to directly lower that variable. Similarly, unfortunately, many people tend to remain in poverty. Second, the cohort was assessed for 15-year mortality risk. It is possible that the risk may have increased if the follow-up period had been longer. However, 15-years among middle aged and older adults would generally capture premature mortality risk.

In conclusion, inflammation and poverty are well known risk factors for mortality, but when both exist simultaneously and CRP is >1.0 mg/dL, they have the potential to increase mortality more than one would expect from an additive effect. This is particularly concerning in socially disadvantaged patients who are already a medically vulnerable population. Moreover, elevated inflammation is not typically known in asymptomatic populations. Perhaps targeted screening for elevated CRP in vulnerable populations might be particularly useful. Even though both inflammation and poverty are modifiable risk factors, in clinical practice, chronic diseases associated with inflammation like cardiovascular disease are more likely to be prevented by healthy lifestyle than be reversed.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: <https://www.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=1999>.

Author contributions

AM: Conceptualization, Project administration, Writing – original draft. FO: Conceptualization, Investigation, Writing – review & editing. LY: Formal analysis, Writing – review & editing. PS: Formal analysis, Writing – review & editing. VW: Conceptualization, Writing – review & editing. AS: Conceptualization, Writing – review & editing.

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Exploring the causal relationship between inflammatory cytokines and immunoinflammatory dermatoses: a Mendelian randomization study

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Objectives: Previous studies have shown that the onset and progression of several immunoinflammatory dermatoses are closely related to specific immune-inflammatory responses. To further assess the causal relationship between 41 inflammatory cytokines and immunoinflammatory dermatoses, we used a Mendelian randomization method.

Methods: Mendelian two-sample randomization utilized inflammatory cytokines from a GWAS abstract containing 8,293 healthy participants as well as psoriasis (4,510 cases and 212,242 controls), atopic dermatitis (7,024 cases and 198,740 controls), and vitiligo (131 cases and 207,482 controls). The causal relationship between exposure and outcome was explored primarily using inverse variance weighting. In addition, multiple sensitivity analyses, including MR-Egger, weighted median, simple model, weighted model, and MR-PRESSO, were simultaneously applied to enhance the final results.

Results: The results showed that in clinical practice, IL-4 and IL-1RA were suggestive indicators of atopic dermatitis risk (OR = 0.878, 95% CI = 0.78–0.99, $p = 0.036$; OR = 0.902, 95% CI = 0.82–1.00, $p = 0.045$). SCGF-b was a suggestive indicator of psoriasis risk (OR = 1.095, 95% CI = 1.01–1.18, $p = 0.023$). IL-4 is a suggestive indicator of vitiligo risk (OR = 2.948, 95% CI = 1.28–6.79, $p = 0.011$).

Conclusion: Our findings suggest that circulating inflammatory cytokines may play a crucial role in the pathogenesis of chronic skin inflammation. IL-4 and IL-1RA may have inhibitory roles in the risk of developing atopic dermatitis, while SCGF-b may have a promoting role in the risk of developing psoriasis. Furthermore, IL-4 may contribute to the risk of developing vitiligo. These results provide insights into further understanding the mechanisms of chronic skin inflammation and offer new targets and strategies for the prevention and treatment of related diseases.

KEYWORDS

immunoinflammatory dermatoses, biomarkers, Mendelian randomization, GWAS, inflammation

Introduction

Immunoinflammatory dermatoses, including psoriasis, atopic dermatitis, and vitiligo, are prevalent clinical skin disorders characterized by immune dysfunction and the infiltration of inflammatory cells in the affected skin areas (1–3). The development and progression of these conditions are associated with aberrant activation of the immune system and the persistence of inflammatory responses. Psoriasis is a chronic inflammatory skin disease characterized by congenital and acquired immune abnormalities (4, 5), hyperproliferation, and aberrant differentiation of epidermal keratinocytes (6) and is often associated with arthritis or cardiometabolic disease (7, 8). In addition, proteomic characterization of psoriatic lesions reveals dermal fibroblast dysfunction, up-regulation of inflammatory cytokines, and signaling or down-regulation of structural molecules (9, 10). Skin inflammation in psoriasis may harbor certain intestinal bacteria, such as *Staphylococcus aureus* and *Streptococcus* daniels, which can exacerbate skin inflammation (11). Atopic dermatitis is an important chronic or recurrent inflammatory skin disease that usually precedes asthma and allergic diseases (12, 13). New insights into the genetics and pathophysiology of atopic dermatitis point to abnormalities in the structure of the epidermis, as well as immune dysregulation, as playing an important role not only in the development of this skin disease but also in asthma and allergy (14). The exact pathogenesis of vitiligo remains elusive and ample evidence exists to suggest changes in the immune process in vitiligo, especially in chronic and progressive diseases (15, 16). The immune system's innate and adaptive immunity appear to be involved as either primary events or secondary outcomes.

Inflammatory cytokines play a crucial role in the pathogenesis of chronic inflammatory skin diseases (17). A better understanding of the inflammatory pathways involved could lead to targeted therapies. The abnormal activation of the immune system leads to the excessive accumulation of immune cells and the release of inflammatory factors. Within these diseases, specific immune cells such as T cells, B cells, and macrophages become activated and aggregate in the affected skin regions, thereby releasing a diverse array of inflammatory cytokines, including growth factors, chemokines, and interleukins.

Inflammatory cytokines also play a crucial role in the immune response. Inflammatory cytokines exert their influence on immune cell function and the development of inflammatory responses by binding to receptors on the surface of immune cells and activating intricate signaling pathways. Research has demonstrated that TNF- α plays a role in the formation of inflammatory skin lesions, stimulating the production of inflammatory mediators and increasing vascular permeability (18). L-17 promotes the proliferation of keratinocytes and the infiltration of inflammatory cells (19). IL-23 is closely associated with IL-17 and enhances IL-17 production through the activation of T-cells and immune cells (19, 20). IL-31 is implicated in the development of skin itching (21). These inflammatory cytokines, along with the pathways they engage, play a significant role in the development of inflammatory skin diseases. Furthermore, the immune-inflammatory response involves other molecules and pathways. For instance, inflammatory mediators and cytokines can activate the nuclear transcription factor NF- κ B, which exhibits increased activity in chronic skin inflammation, resulting in heightened expression of inflammatory genes (22). Additionally, recent studies have indicated the

involvement of immune cells such as T cells and dendritic cells in the initiation and progression of chronic skin inflammation (23, 24).

In this study, for the first time, we extracted validated genetic variants from published genome-wide association study (GWAS) pooled data for 41 inflammatory cytokines to investigate their association with three autoimmune dermatoses. Mendelian random (MR) analysis methods utilize genetic variation in non-experimental data to infer causal effects of exposure on outcomes. Because alleles are randomly assigned during meiosis, MR reduces traditional confounding variables and reverse causation, thus providing better evidence for causal inference (25). Two-sample MR analyses allow researchers to assess associations between instrument exposure and instrument outcome in two independent population samples, thereby improving the applicability and validity of the test.

Method

Mendelian randomization

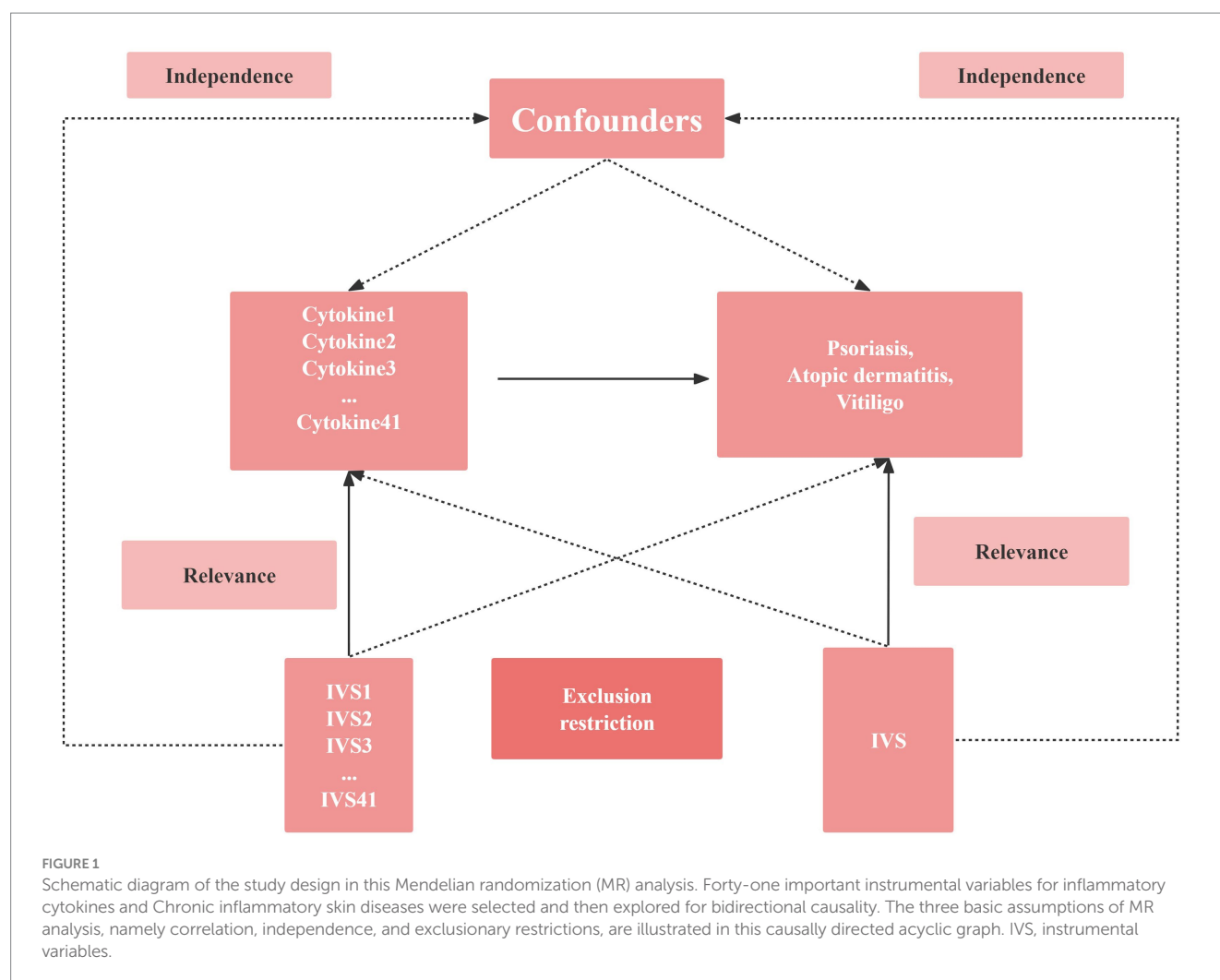
Mendelian randomization is an analytical method used to assess causal relationships between observed modifiable exposures or risk factors and clinically relevant outcomes. Genome-wide association studies (GWAS) have identified tens of thousands of common genetic variants that are associated with hundreds of complex traits (26). This provides a valuable tool for studying causality, especially when randomized controlled trials are not feasible or when observational studies are subject to confounding or reverse causation leading to association bias. To address these issues, Mendelian randomization uses genetic variants as instrumental variables for testing exposure. These exposure-associated alleles of genetic variants are randomly assigned and are not subject to reverse causation. Due to the wide availability of published genetic associations, screening for appropriate genetic instrumental variables makes Mendelian randomization a time- and cost-effective method and is becoming increasingly popular for assessing and screening for potential causal associations. The observed associations between genetic instrumental variables and outcomes support the hypothesis that there is a causal relationship between the exposures and outcomes discussed. This approach helps to overcome the difficulty of conducting randomized controlled trials while mitigating association bias in observational studies due to confounding or reverse causation. Thus, Mendelian randomization provides a powerful tool for studying complex traits and potential causal relationships (27).

Data resources

The study design included atopic dermatitis cases from a meta-analysis study that included 7,024 cases and 198,740 controls of European ancestry¹; psoriasis cases from a meta-analysis study that included 4,510 cases and 212,242 controls of European ancestry², and vitiligo cases from a meta-analysis study that included 131 cases and

1 Trait: Atopic dermatitis - IEU OpenGWAS project (mrcieu.ac.uk)

2 Trait: Psoriasis - IEU OpenGWAS project (mrcieu.ac.uk)



207,482 controls of European ancestry (Figure 1).³ For the genetic instrument of cytokines, summary statistics were taken from the most comprehensive and extensive cytokine GWAS; the GWAS cytokine meta-analysis included 8,293 Finns from three independent population cohorts: the Young Finns Cardiovascular Risk Study, the FINRISK 1997 and the FINRISK 2002 studies (28). The survey was conducted in Finland, with participants aged 25 to 74 years randomly selected from five different geographic regions. Cytokine levels were measured in the participants' EDTA plasma, heparin plasma and serum. Only measurements within the detectable range of each cytokine were included in the analysis, and any cytokines missing more than 90% of their values (48 of 7) were excluded. All participants provided written informed consent.

Selection of cytokine SNPs

MR analysis has three core assumptions, namely correlation, independence, and exclusion restriction (29). It is assumed that the

selected genetic variants are associated with risk factors (correlation) but not with any confounders in the risk factor-outcome association (independence) and that they are not associated with the outcome through any pathway other than the risk factor of interest (exclusion restriction). In this two-way study, four GWAS, 41 inflammatory cytokines and three autoimmune dermatoses were utilized. First, we used $p < 5 \times 10^{-8}$ as a genome-wide significance threshold to select SNPs strongly associated with three autoimmune dermatoses and inflammatory cytokines. Second, to avoid linkage disequilibrium, we clustered these SNPs ($kb = 10,000$, $r^2 = 0.001$). Palindromic SNPs were discarded because we could not identify these SNPs in exposure and outcome of GWASs in systemic inflammatory regulators were aligned in the same direction. Third, the R^2 value of each SNP was used to calculate the proportion of variance in exposure, and the F statistic was used to estimate the instrumental strength to avoid weak instrumental bias (30, 31). Finally, we will replace the unavailable SNPs in the result summary with the proxy SNPs ($R^2 > 0.8$) from LDlink.⁴

³ Trait: Vitiligo - IEU OpenGWAS project (mrcieu.ac.uk)

⁴ LDlink|An Interactive Web Tool for Exploring Linkage Disequilibrium in Population Groups (nih.gov)

Statistical analysis

Since each cytokine has a different number of SNPs, we chose the Wald ratio as the primary MR analysis in cytokines with only one SNP. We chose inverse variance weighted (IVW) as the primary MR analysis in people with two or more SNPs, to assess the potential pathogenic role of inflammatory cytokines and the risk of autoimmune dermatoses. Subsequently, we performed a Cochrane Q test on IVW to detect heterogeneity. No heterogeneity was observed for most outcomes, with p values greater than 0.05. Only a few showed heterogeneity, but our primary MR analysis was IVW; heterogeneity can be present in it, so the presence of heterogeneity in individual outcomes does not have much impact on the prediction of causality.

Next, to further assess causality and investigate the presence of pleiotropy, we performed a set of checks, including MR Egger regression and MR-PRESSO. In addition, Leave-one-out was used to analyze the possibility of individual SNPs confounding the overall MR analysis. We also used PhenoScanner to examine potential dimorphic phenotypes in the evaluated individual SNPs to eliminate their potential influence on the results. Most of the above work was performed in R analysis software (version 4.0.3) for the relevant R packages, including two sample MRs, data arrays, etc.

Result

Details of the study and dataset are in [Supplementary Table S1](#), the participants were all European (100%), overcoming ethnic differences.

Causal relationship between IL-4 and IL-1RA and atopic dermatitis

Our findings suggest a potential role for IL-4 and IL-1RA in the risk of developing atopic dermatitis according to the IVW method ([Figure 2](#)). By using the IVW method, we found that higher levels of IL-4 and IL-1RA genetic prediction were associated with a lower risk of atopic dermatitis, OR = 0.878, 95% CI = 0.78–0.99, p = 0.036 per 1 standard deviation (SD); OR = 0.902, 95% CI = 0.82–1.00, p = 0.045 per 1 standard deviation (SD) ([Figures 3A,B,E,F](#)). Using Cochran's Q test, we also did not observe heterogeneity (p = 0.478; p = 0.939), nor did we find directional polymorphism (MR egger-intercept = 0.002, p = 0.892 for MR egger-intercept; p = 0.48 for MR PRESSO global test; MR egger-intercept = -0.015, P for MR egger-intercept = 0.489; P for MR PRESSO global test = 0.947). Except for IL-4 and IL-1RA, none of the other cytokines (e.g., VEGF, GRO- α , Trail, MIG, IL-7, IL-17) were shown to be associated with the risk of osteonecrosis in the IVW primary MR analysis or other secondary analyses. In the heterogeneity test, we found significant heterogeneity for SCF (p = 0.001), MIG (p = 0.002), FGFBasic (p = 0.008), IL-5 (p = 0.021), MIP1b (p = 0.027), and GCSF (p = 0.037), whereas the majority of the other cytokines demonstrated significant non-heterogeneity. Our MR-egger regression did not find any polymorphism in the p -values of all cytokines except IL-2 (p = 0.022 for MR egger-intercept). Finally, our MR-PRESSO assay as an additional robustness test did not find any outliers except for MIG (p = 0.01), FGFBasic (p = 0.016), IL-5 (p = 0.024) and MIP1b (p = 0.037; [Supplementary Tables S1–S3](#)).

Causal relationship between SCGF-b and psoriasis

In the present study, based on the IVW approach, we identified a potential role of SCGF-b in the risk of psoriasis development ([Figure 4](#)). By using the IVW method, we found that higher levels of SCGF-b genetic prediction were associated with a higher risk of psoriasis, (OR = 1.095, 95% CI = 1.01–1.18, p = 0.023 per 1 standard deviation (SD); [Figures 3C,G](#)). Using Cochran's Q test, we also did not observe heterogeneity (p = 0.478), nor did we find directional polymorphism (MR egger-intercept = 0.015, p = 0.349 for MR egger-intercept; p = 0.538 for MR PRESSO global test). Except for SCGF-b, none of the other cytokines (e.g., VEGF, GRO- α , Trail, MIG, IL-7, IL-17) were shown to be associated with the risk of osteonecrosis in the IVW primary MR analysis or other secondary analyses. In the heterogeneity test, we found significant heterogeneity for GROa (p = 0.001), FGFBasic (p = 0.007), IL-9 (p = 0.016), and HGF (p = 0.021), whereas most of the other cytokines showed significant non-heterogeneity. Furthermore, our MR-egger regression did not reveal any polymorphism in the p -values of all cytokines. Except for GROa (p = 0.003), our MR-PRESSO assay as an additional robustness test did not find any outliers ([Supplementary Tables S4–S6](#)).

Causal relationship between IL-4 and vitiligo

By using the IVW method, we found that higher levels of IL-4 genetic prediction were associated with a higher risk of vitiligo, (OR = 2.948, 95% CI = 1.28–6.79, p = 0.011 per 1 standard deviation (SD); [Figure 5; Figures 3D,H](#)). Using Cochran's Q test, we also did not observe heterogeneity (p = 0.836), nor did we find directional polymorphism (MR egger-intercept = 0.059, p = 0.592 for MR egger-intercept; p = 0.842 for MR PRESSO global test). Except for IL-4, none of the other cytokines (e.g., VEGF, GRO- α , Trail, MIG, IL-7, IL-17) were shown to be associated with the risk of osteonecrosis in the IVW primary MR analysis or other secondary analyses. In the heterogeneity test, we found significant heterogeneity for IL-13 (p = 0.037), while most of the other cytokines showed significant non-heterogeneity. In addition, our MR-egger regression did not find any polymorphism in the p -values of all cytokines. Finally, our MR-PRESSO assay as an additional robustness test did not reveal any outliers except for IL-13 (p = 0.044) ([Supplementary Tables S7–S9](#)).

Discussion

Inflammatory factors play a pivotal role in the development of autoimmune dermatoses. Abnormal activation of the immune system triggers the accumulation of immune cells and the release of inflammatory factors. The release of these inflammatory mediators initiates a persistent inflammatory response, leading to abnormal changes in the skin tissue, including abnormal keratinization, impaired epidermal barrier function, and vasodilation ([17, 23](#)). This vicious cycle further stimulates the release of immune cells and inflammatory factors, exacerbating the persistence of chronic inflammation. In this study, we employed MR analysis methods to investigate the potential role of circulating cytokines in the risk of

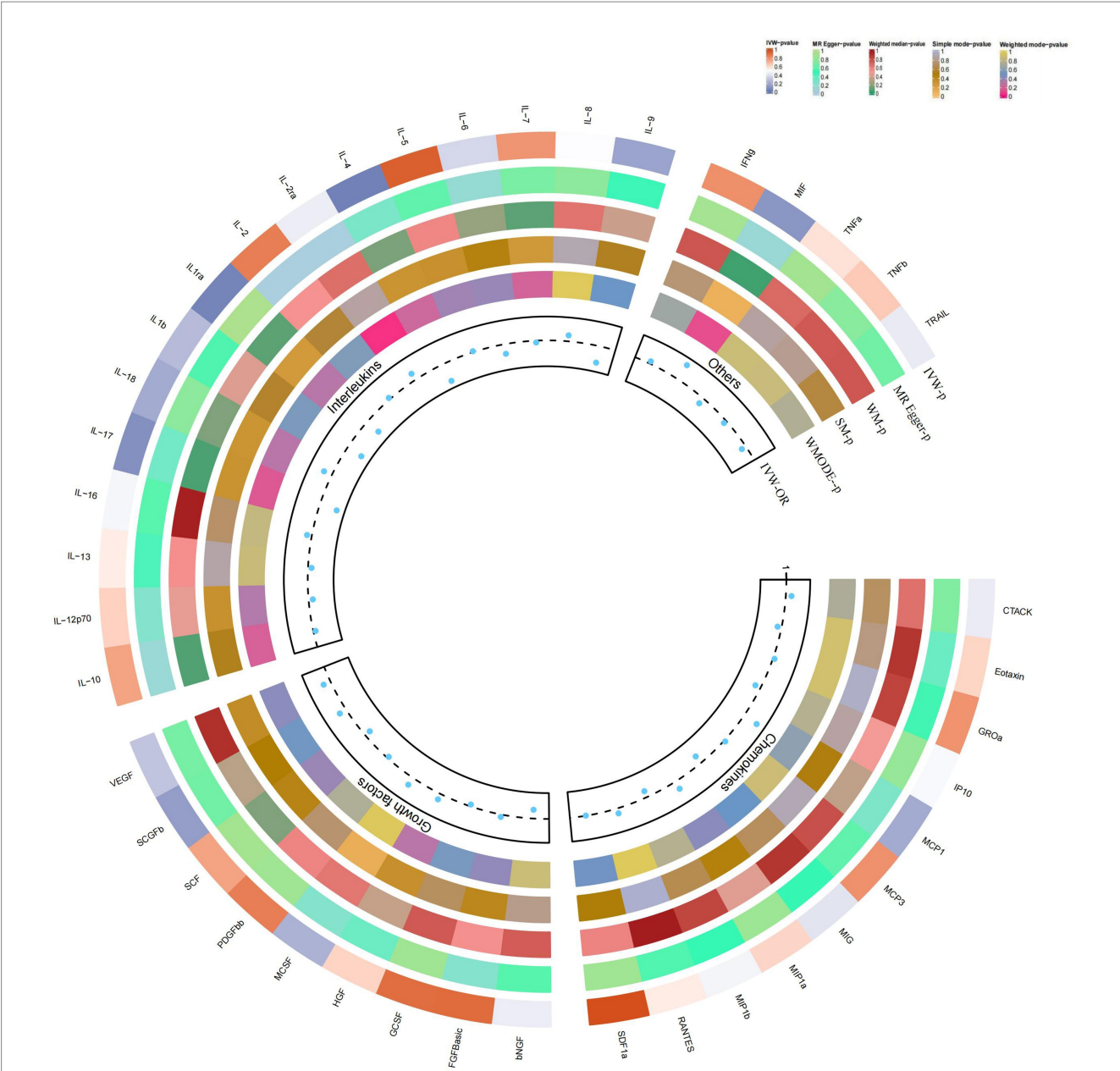


FIGURE 2
Causal correlations of 41 inflammatory cytokines on atopic dermatitis. The change in the odds ratio (OR) of atopic dermatitis per one-SD rise in the cytokine level is shown by OR and 95% confidence interval. p -value $0.05/41 = 0.0012$ was found significant after multiple-comparison correction. The results from the inverse variance weighted method were shown for all cytokines. bNGF, beta nerve growth factor; CTACK, cutaneous T cell-attracting chemokine; FGFBasic, basic fibroblast growth factor; GCSF, granulocyte colony-stimulating factor; GROa, growth-regulated oncogene-a; HGF, hepatocyte growth factor; IFNg, interferon gamma; IL, interleukin; IP, interferon gamma-induced protein 10; MCP1, monocyte chemotactic protein 1; MCP3, monocyte-specific chemokine 3; MCSF, macrophage colony-stimulating factor; MIF, macrophage migration inhibitory factor; MIG, monokine induced by interferon gamma; MIP1a, macrophage inflammatory protein-1a; MIP1b, macrophage inflammatory protein-1b; PDGFbb, platelet-derived growth factor BB; RANTES, regulated upon activation normal T cell expressed and secreted factor; SCF, stem cell factor; SCGFb, stem cell growth factor beta; SDF1a, stromal cell-derived factor-1 alpha; SNPs, single-nucleotide polymorphisms; TNFa, tumor necrosis factor alpha; TNFb, tumor necrosis factor beta; TRAIL, TNF-related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor*.

developing these diseases, focusing specifically on the causal relationship between circulating cytokines and atopic dermatitis, psoriasis, and vitiligo.

Several studies have now revealed the potential role of IL-1RA in the etiology and treatment of acne (32, 33). Our study provides new proof of the above ideas in a genetic perspective. Using the IVW method, we observed that higher genetic prediction levels of IL-1RA

were associated with a lower risk of atopic dermatitis. The mechanisms and pathways involved in the risk reduction of IL-1RA in atopic dermatitis are complex. IL-1RA is an antagonist of IL-1 and inhibits IL-1 activity by competitively binding to the IL-1 receptor (34). Interleukin (IL)-1 family cytokines initiate inflammatory responses and modulate innate and adaptive immunity. While they play a crucial role in host defense, excessive immune activation can also lead to the

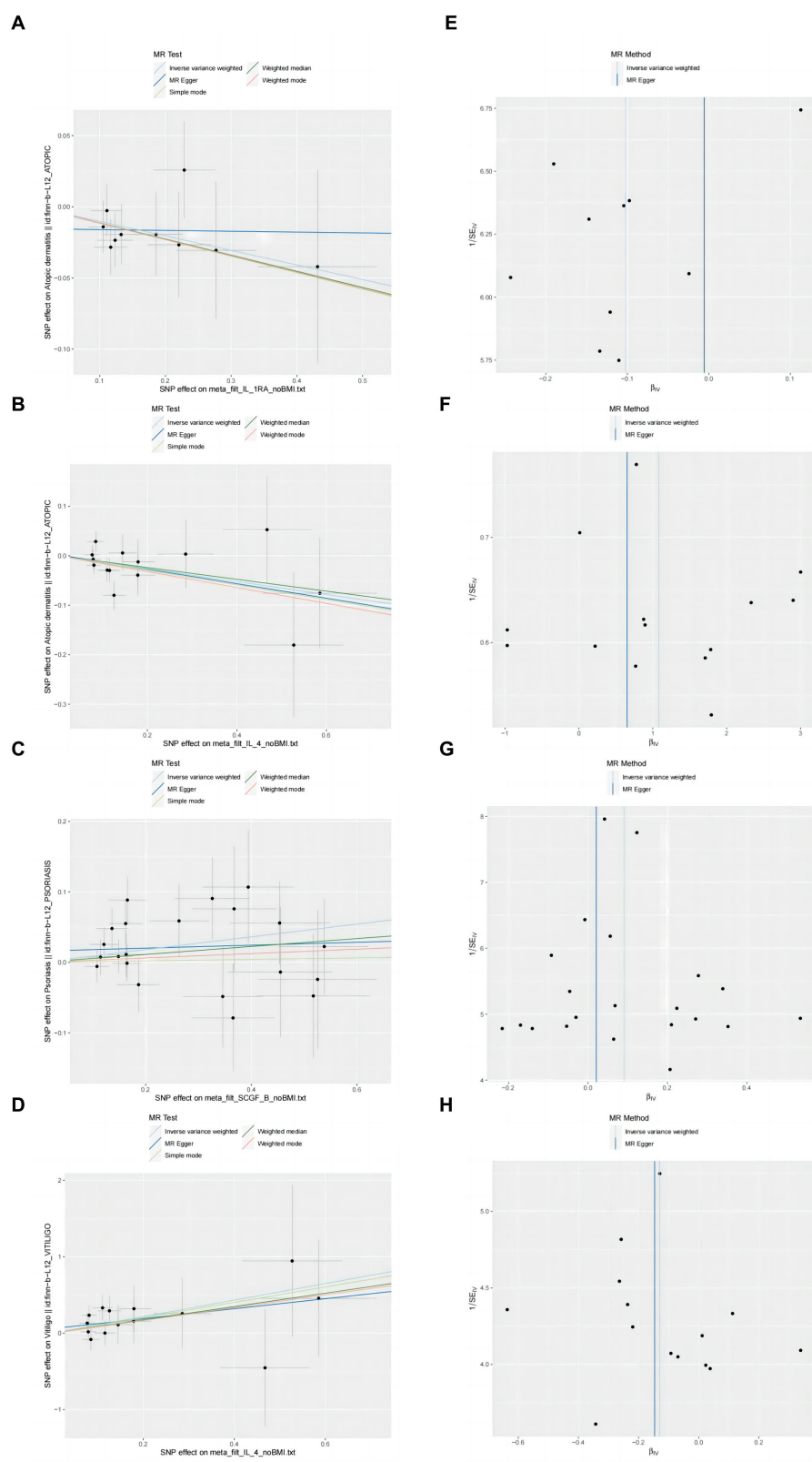


FIGURE 3

Scatter plots and funnel plots of MR analyses for IL-1RA IL-4, SCGF-b and IL-4 in psoriasis, atopic dermatitis, vitiligo. (A–D) Individual inverse variance (IV) associations with cytokine risk are displayed versus individual IV associations with Chronic inflammatory skin diseases in black dots. The 95%CI of odd ratio for each IV is shown by vertical and horizontal lines. The slope of the lines represents the estimated causal effect of the MR methods. (E–H) The funnel plots show the inverse variance weighted MR estimate of each cytokine single-nucleotide polymorphism with Chronic inflammatory skin diseases versus 1/standard error (1/SEIV).

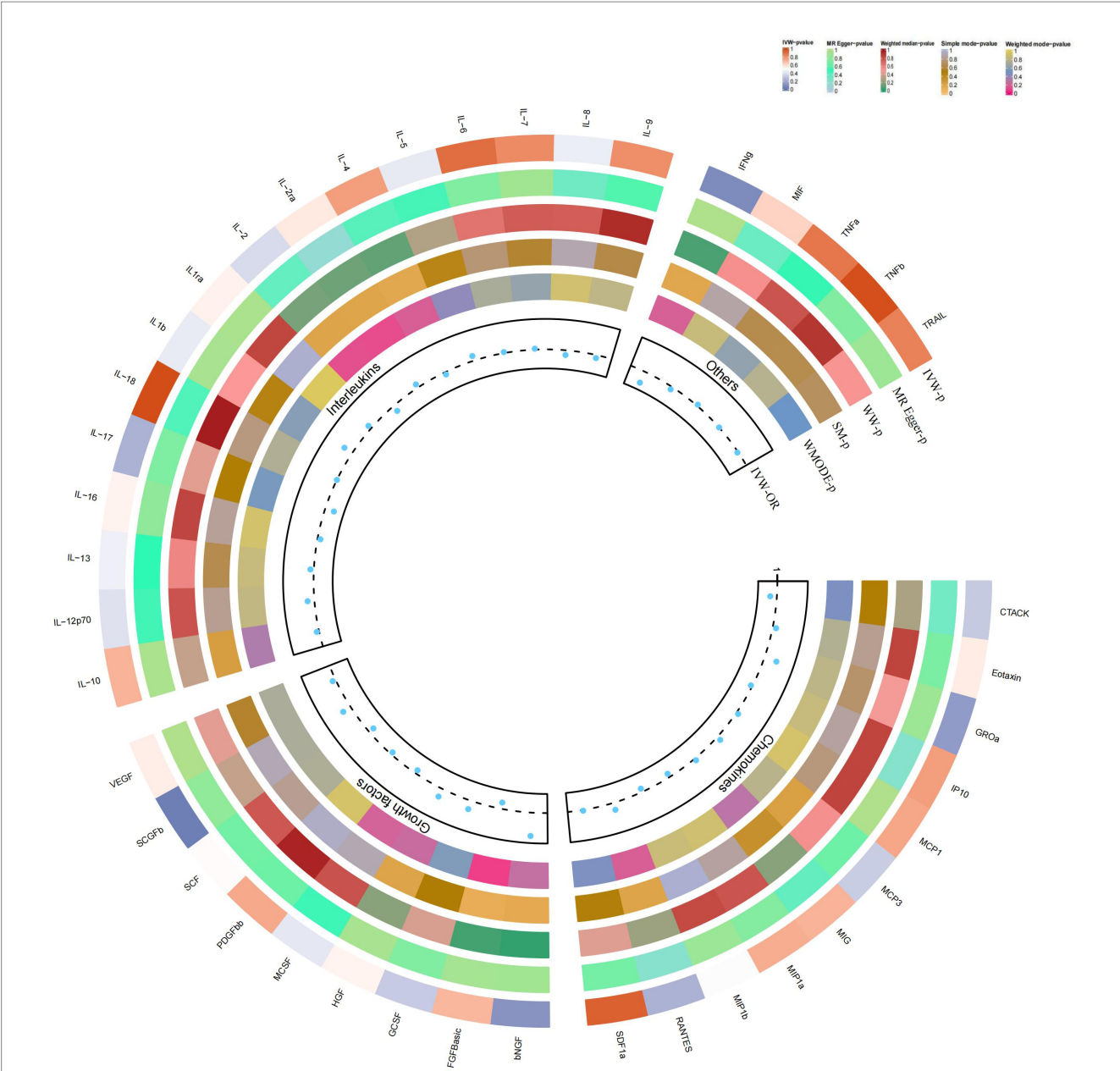


FIGURE 4
Causal correlations of 41 inflammatory cytokines on psoriasis. The change in the odds ratio (OR) of psoriasis per one-SD rise in the cytokine level is shown by OR and 95% confidence interval. p -value $0.05/41 = 0.0012$ was found significant after multiple-comparison correction. The results from the inverse variance weighted method were shown for all cytokines.

development of chronic inflammatory diseases (35). By inhibiting the signaling pathway of IL-1, IL-1RA reduces the release of inflammatory mediators and the activation of inflammatory responses. Studies have shown that IL-1RA reduces inflammatory responses and skin damage by inhibiting the activity of IL-1 (36, 37). These cytokines interact with each other through various mechanisms, jointly regulating the pathogenesis of atopic dermatitis. The combined findings suggest that the reduced risk of atopic dermatitis associated with IL-1RA may be realized through multiple pathways, including immune regulation, inflammatory modulation, and restoration of skin barrier function.

SCGFb (Stem Cell Growth Factor Beta) is a newly discovered secreted sulfated glycoprotein that functions as a growth factor in

early hematopoiesis (38). It is selectively produced by bone and hematopoietic stromal cells and can mediate its proliferative activity on primitive hematopoietic progenitor cells (39). Several studies have shown that SCGF-b is causally associated with a variety of immune-related diseases, but the subsequent mechanisms have not been revealed (40–42). Nevertheless, this novel biomarker may also complement the limitations of conventional biomarkers in routine clinical practice.

Psoriasis is an immune-mediated chronic skin disease characterized by the aberrant activation of multiple immune cells and inflammatory factors (43, 44). Currently, no studies have revealed a potential link between SCGF-b and psoriasis. However,

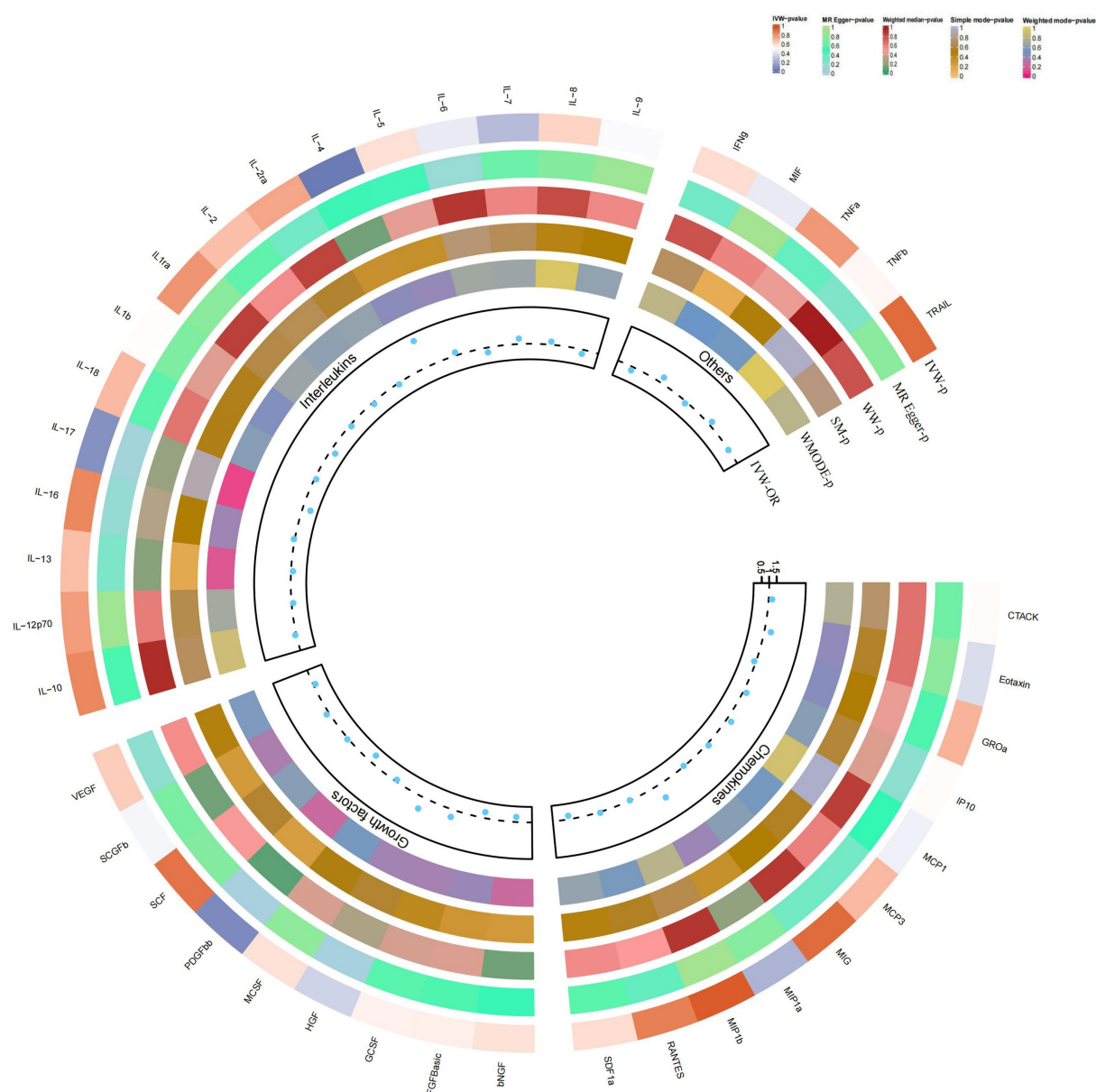


FIGURE 5

Causal correlations of 41 inflammatory cytokines on vitiligo. The change in the odds ratio (OR) of vitiligo per one-SD rise in the cytokine level is shown by OR and 95% confidence interval. p -value $0.05/41 = 0.0012$ was found significant after multiple-comparison correction. The results from the inverse variance weighted method were shown for all cytokines.

our study found that SCGF-b may be associated with an increased risk of psoriasis development, with higher genetic prediction levels of SCGF-b being associated with a higher risk. Numerous studies have shown that abnormal proliferation and differentiation of stem cells are closely related to the development of psoriasis and are involved in the immunomodulatory process of the disease (45–47). Furthermore, SCGF-b has been associated with the prognosis of Crohn's disease (CD), and considering the potential causal relationship between CD and psoriasis, SCGF-b may also have a role in psoriasis (48, 49). A comprehensive understanding of the function and regulatory mechanisms of SCGF-b can help uncover the pathophysiological mechanisms of psoriasis and provide new targets and strategies for its treatment and intervention.

Finally, we found that IL-4 had important associations with the risk of immunoinflammatory dermatoses. First, higher genetically predicted levels of IL-4 were associated with a higher risk of vitiligo. This suggests that IL-4 may contribute to the pathogenesis of vitiligo. IL-4 is involved in immunomodulation and affects melanocyte function and proliferation by regulating the JAK2-STAT6 pathway (50). Additionally, IL-4 promotes the inflammatory response by stimulating the proliferation and activation of inflammatory cells, leading to an inflammatory response (51). The infiltration of inflammatory cells and the release of inflammatory mediators can cause damage to surrounding melanocytes, exacerbating the onset of pigmentary disorders (52, 53). Elevated levels of IL-4 may affect the activation status of immune cells, leading to increased attack on melanocytes and further promoting the development of vitiligo (54).

Some IL-4 findings in vitiligo studies seem to be contrary to ours (55, 56). We believe that there may be differences in experimental design and methodology between the previous studies and ours. This includes aspects such as sample selection, analytical techniques, and experimental conditions. In addition, biological differences between different patient populations may influence the expression of IL-4 levels. Previous studies and our study may involve different patient groups, which may be one of the reasons for the conflicting results. Vitiligo may undergo different inflammatory stages, and IL-4 expression levels may vary between these stages. Previous studies and our study may have targeted different stages of the vitiligo disease process, leading to inconsistent results. A comprehensive understanding of the function and regulatory mechanisms of IL-4 can help unravel the pathophysiological mechanisms of vitiligo and provide new targets and strategies for its treatment and intervention.

Furthermore, while it is noteworthy that higher IL-4 levels in our study were associated with a lower risk of atopic dermatitis, several studies have demonstrated the involvement of IL-4 in the onset and development of atopic dermatitis (57, 58). Our study may reveal additional pathways through which IL-4 is involved in atopic dermatitis. IL-4, a cytokine secreted by Th2 cells, regulates immune responses and inflammatory processes by activating its receptors (57). It inhibits the Th1 immune response, reduces the infiltration of inflammatory cells and inflammatory responses, and promotes the production of the anti-inflammatory cytokine IL-10 (59). Additionally, IL-4 and IL-1RA act by regulating inflammation-related molecules and pathways. IL-4 inhibits the production of multiple inflammatory mediators, such as IL-17, IL-22, and TNF- α , while promoting skin barrier repair and protection.

It is necessary to point out some limitations of this study. First, our study used MR analysis to infer causality, but was unable to consider potential confounding factors. For example, we could not know whether the patients in this study had other diseases that could cause inflammation. Therefore, our results need to be validated in larger studies. Second, we only considered the role of a few cytokines, while other unexplored cytokines may also play a key role in chronic skin inflammation. Third, MR analysis can only be used to infer causality and cannot yet elaborate the causal relationship between the study population and disease severity or duration, and further studies could investigate a broader network of cytokines and their interactions. Fourth, individual differences, different disease stages, and patient treatment history may all contribute to fluctuations in cytokine (IL-4) levels. There may be complex networks of interactions with other cytokines that may also have an impact on the results. Fifth, instrumental variables from different analytical platforms, experiments, populations, etc. may be heterogeneous, thus affecting the results of Mendelian randomization analysis. Finally, we must point out that the two-sample Mendelian randomization method also has some limitations. In two-sample Mendelian randomization, the dataset used to perform the Mendelian randomization is the same as the dataset used to identify the instruments. This can lead to the phenomenon of the winner's curse, i.e., GWAS data can lead to an overestimation of genetic effect sizes (60, 61). If the instruments in GWAS are not sufficiently accurate, then the results of MR may be biased, thus affecting the accuracy of causality. In addition, since we were unable to provide the original dataset prior to harmonization, this could lead to bias due to improper data harmonization (62).

In summary, our findings suggest that circulating inflammatory cytokines may play a crucial role in the pathogenesis of chronic skin

inflammation. IL-4 and IL-1RA may have inhibitory roles in the risk of developing atopic dermatitis, while SCGF-b may have a promoting role in the risk of developing psoriasis. Furthermore, IL-4 may contribute to the risk of developing vitiligo. Studies have shown that changes in cytokine levels caused by targeted therapies alter disease symptoms (63, 64). Our results provide insight into further understanding the mechanisms of chronic skin inflammation and offer new targets and strategies for the prevention and treatment of immunoinflammatory dermatoses.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding author.

Author contributions

JL: Data curation, Software, Writing – original draft. YL: Conceptualization, Writing – original draft. XZ: Conceptualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmed.2024.1263714/full#supplementary-material>

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Systemic immune-inflammatory biomarkers (SII, NLR, PLR and LMR) linked to non-alcoholic fatty liver disease risk

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Background: Systemic immune-inflammatory biomarkers including systemic immune inflammation index (SII), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and lymphocyte-to-monocyte ratio (LMR) have been demonstrated to be associated with the risk and severity of various liver diseases. However, studies on their role and clinical significance in metabolic diseases, especially in nonalcoholic fatty liver disease (NAFLD), are limited and results are inconsistent.

Methods: 10821 adults aged 20 years or older were enrolled in this cross-sectional study, sourced from six cycles of the National Health and Nutrition Examination Survey (NHANES). Survey-weighted logistic regression was employed to investigate the correlation between systemic immune-inflammatory biomarkers (SII, NLR, PLR, and LMR) and NAFLD risk. Restricted cubic spline regression models and segmented regression models were used to describe nonlinear relationships and threshold effects. Subgroup and sensitivity analyses were also conducted.

Results: After adjusting for all confounding variables, there was a significant positive association observed between ln-transformed SII (OR= 1.46, 95% CI: 1.27-1.69, $P < 0.001$), NLR (OR= 1.25, 95% CI: 1.05-1.49, $P = 0.015$), LMR (OR= 1.39, 95% CI: 1.14-1.69, $P = 0.002$) with NAFLD. A nonlinear dose-response relationship with an inverted "U"-shaped threshold of 4.64 was observed between ln(PLR) and NAFLD risk. When ln(PLR) was below 4.64, each unit increase in ln(PLR) was associated with a 0.55-fold increase in the risk of NAFLD (OR= 1.55, 95% CI: 1.05-2.31, $P < 0.05$). Conversely, when ln(PLR) exceeded 4.64, each unit increase in ln(PLR) was associated with a 0.40-fold decrease in the risk of NAFLD (OR= 0.60, 95% CI: 0.44-0.81, $P < 0.05$).

Conclusion: ln-transformed SII, NLR, and LMR were linearly associated with NAFLD risk. ln(PLR) showed an inverted “U”-shaped nonlinear dose-response relationship with the risk of NAFLD.

KEYWORDS

NAFLD, systemic immune-inflammatory biomarkers, NHANES, population-based study, metabolic disease

1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases, characterized by the presence of liver fat deposition in more than 5% of hepatocytes, unrelated to excessive alcohol consumption (1, 2). It includes non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH), with NASH being prone to progression to liver fibrosis and potentially leading to severe complications such as liver cirrhosis, hepatocellular carcinoma, and liver failure (1, 2). NAFLD affects over 25% of the global population, with its incidence continuing to rise, making it a significant public health issue worldwide and imposing a substantial socioeconomic burden (3). It is estimated that the total population affected by NAFLD will increase by 18.3% by 2030 (4). However, awareness of the disease remains limited, as more than 95% of adult NAFLD patients are unaware of their condition (5). Cardiovascular disease (CVD) is the leading cause of mortality in NAFLD patients (6). NAFLD leads to various extrahepatic complications and is closely associated with metabolic cardiovascular risk factors such as obesity, insulin resistance, type 2 diabetes (T2DM), metabolic syndrome, hypertension, and dyslipidemia, further increasing the risk of CVD and long-term morbidity and mortality (6–8).

Although the incidence and potential risks of NAFLD are high, the pathogenesis of this disease remains incompletely understood, and there are currently no standardized and universally accepted non-invasive diagnostic methods (1, 2). NAFLD patients typically do not exhibit symptoms or may only experience fatigue and vague discomfort in the right upper abdomen, often detected through abnormal liver biochemistry or imaging examinations (9). However, studies on patients with T2DM have shown that a considerable proportion of them have normal plasma transaminase levels, even among those with clinically significant fibrosis (F2–4), with most plasma transaminase levels being below 40 U/L (10, 11). In terms of imaging examinations, ultrasound is not sufficiently sensitive for detecting mild hepatic steatosis (1, 12). However, H-MRS and MRI-PDFF are the most accurate and sensitive in diagnosing hepatic steatosis. However, their use is

currently limited to clinical research due to the high cost involved (13, 14). Liver biopsy remains the gold standard for diagnosis, but its invasive nature, sampling errors, and inherent risks of complications restrict its use in clinical practice (15). Therefore, there is an urgent need to identify new and reliable biomarkers for diagnosing, prognosis, and monitoring NAFLD.

Given the complex interplay between metabolic dysfunction, chronic inflammation, and liver disease, there is increasing interest in the role of systemic inflammation in the development and progression of NAFLD (16, 17). Also, oxidative stress can mediate apoptosis and lead to inflammation by regulating Radical oxygen species (18). During the progression of NASH, there are changes in the composition of immune cells within the liver, along with interactions and disruptions between immune cells and parenchymal cells. Multiple immune cell types are involved in the development of the disease, associated with the severity of hepatic steatosis, fibrosis, inflammation, and cellular injury (19). Systemic immune-inflammatory biomarkers include the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and lymphocyte-to-monocyte ratio (LMR), which reflect the balance of immune response and the overall inflammatory environment (20, 21). Additionally, the systemic immune inflammation index (SII) is a comprehensive novel biomarker of inflammation that reflects both localized immune responses and the overall level of inflammation in the body (22). Previous studies have reported associations between these immune-inflammatory markers and the risk and severity of various liver diseases, such as viral hepatitis, cirrhosis, and hepatocellular carcinoma (23, 24). However, there is limited research and inconsistent results regarding the role and clinical significance of SII, NLR, PLR, and LMR in NAFLD (20, 21, 25).

Therefore, The primary objective of this study was to conduct a comprehensive investigation into the association between systemic immune-inflammatory biomarkers (SII, NLR, PLR, and LMR) and the risk of NAFLD. Employing a cross-sectional study design, we utilized a substantial and representative sample from the national population to ensure a thorough analysis. The central emphasis of the study was to elucidate the potential of these markers as diagnostic indicators for NAFLD.

2 Methods

2.1 Study design and population

The National Health and Nutrition Examination Survey (NHANES), an ongoing cross-sectional study of national significance in the United States, serves as a crucial source of regular health-related data for the nation. All NHANES studies passed the National Center for Health Statistics (NCHS) Ethics Review Board and written informed consent was obtained from all participants (<https://www.cdc.gov/nchs/nhanes/irba98.htm>). The number of participants in the NHANES survey during the study period determined the sample size. The NHANES surveys encompass a wide array of essential domains including demographics, socioeconomic aspects, dietary patterns, and health-related information. The data collection is orchestrated using a multilevel, complex sampling methodology, further elucidated on the official NHANES website (<https://www.cdc.gov/nchs/index.htm>). Our study recruited 59842 participants from 6 cycles of NHANES (2017–2018, 2015–2016, 2013–2014, 2011–2012, 2009–2010, 2007–2008). To maintain the integrity and validity of our findings, stringent exclusion criteria were applied. Individuals with missing data about alcohol consumption, viral hepatitis status (serum hepatitis B surface antigen and serum hepatitis C antibody data), or essential covariates such as age, sex, ethnicity, waist circumference, fasting glucose levels, and insulin were excluded from the analysis. Pregnant and participants younger than 20 years of age were also excluded. Ultimately, the study included 10821 participants (Supplementary Figure 1).

2.2 Assessment of NAFLD

In this study, NAFLD was defined by a US Fatty Liver Index (USFLI) score exceeding 30, with careful consideration to exclude cases of excessive alcohol consumption (<20 g/day for males and <10 g/day for females) or the presence of viral hepatitis (indicated by a positive serum hepatitis B surface antigen or serum hepatitis C antibody) (26–28). The USFLI score has been validated to have an area under the operating characteristic curve (AUROC) of 0.80 (sensitivity, 62%; specificity, 88%) in diagnosing whether a subject has NAFLD (26).

2.3 Systemic immune-inflammatory biomarkers (SII, NLR, PLR, LMR)

Systemic immune-inflammatory biomarkers derived from complete blood count, including the SII, NLR, PLR, and LMR, have been used as predictors of risk and prognosis for various diseases (29–31). The NHANES Laboratory Procedures Manual (LPM) provides standardized protocols for measuring these biomarkers and explanations of any possible biases, details of which can be found at https://www.cdc.gov/nchs/nhanes/biospecimens/serum_plasma_urine.htm. In the present study, we

sought to comprehensively unravel the correlation between systemic immune-inflammatory biomarkers and NAFLD. To achieve this, we calculated the SII, NLR, PLR, and LMR using the following formulas: $SII = \text{platelet counts} \times \text{neutrophil counts} / \text{lymphocyte counts}$, $NLR = \text{neutrophil counts} / \text{lymphocyte counts}$, $PLR = \text{platelet counts} / \text{lymphocyte counts}$, $LMR = \text{lymphocyte counts} / \text{monocyte counts}$.

2.4 Covariates

Based on both existing literature and clinical insights, we included the following covariates: age, gender, race, family poverty income ratio (PIR), education level, smoking status, body mass index (BMI), diabetes, hypertension, hyperlipidemia, and alanine aminotransferase (ALT) (27, 28, 32). Within the NHANES survey framework, we have categorized race into five categories: Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, and Other Race. PIR was categorized as low (≤ 1.3), medium (1.3–3.5), and high (> 3.5) based on the household poverty income ratio (27). Likewise, educational level was categorized as less than high school, high school or equivalent, and some college or more. Smoking status was determined by NHANES survey questions and participants were defined as smokers if they had smoked at least 100 cigarettes in their lifetime. BMI was categorized as < 18.5 , 18.5–24.9, 25.0–29.9, and ≥ 30.0 kg/m². For diabetes, we adopted a comprehensive definition encompassing a fasting blood glucose level ≥ 126 mg/dL, a hemoglobin A1c $\geq 6.5\%$, use of oral hypoglycemic agents, insulin use, or self-reported history of diabetes (28). Hypertension was defined as a systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg, or a self-reported history of hypertension or oral antihypertensive medications (32, 33). Hyperlipidemia has been defined as serum total cholesterol of 200 mg/dL, triglycerides of 150 mg/dL, high-density lipoprotein (HDL) of 40 mg/dL in men and 50 mg/dL in women, or low-density lipoprotein (LDL) of 130 mg/dL (34).

2.5 Statistical analyses

Continuous variables are expressed as mean (standard deviation) and categorical variables as frequency (percentage). For between-group comparisons of baseline information, weighted t-tests were used for continuous variables and weighted chi-square tests for categorical information. Since the SII, NLR, PLR, and LMR distributions were skewed, a logarithmic transformation was applied using natural logarithm (ln) to achieve an approximately normal distribution, which was then stratified into quartiles (Q1, Q2, Q3, and Q4).

First, multifactorial logistic regression was employed to analyze the influence of SII, NLR, PLR, and LMR on the risk of NAFLD. At the same time, ln-transformed SII, NLR, PLR, and LMR were considered as categorical variables (quartiles) for sensitivity analysis, and multifactorial logistic regression was repeated, with

the lowest quartile (Q1) as the reference group, and the results were expressed as ratio ratios (95% confidence intervals). A trend test was also conducted. In our study, we constructed 3 models by adjusting for different confounding variables. The crude model remained unadjusted, while model 1 was adjusted for age, gender, and race. Model 2 was adjusted for all covariates based on model 1. Second, to address potential nonlinear relationships between SII, NLR, PLR, LMR, and NAFLD risk, restricted cubic spline (RCS) regression was performed. The likelihood ratio test was used to test for nonlinearity. When a nonlinear relationship was detected, a two-stage segmented regression was carried out using the inflection point values to explore the threshold effects of the independent variables on NAFLD. Further, to examine whether this relationship was modified by age, gender, race, household poverty income, education, BMI, hypertension, and hyperlipidemia, we conducted interaction analyses and subgroup analyses considering SII, NLR, PLR, and LMR as continuous and categorical variables (quartiles), respectively. Finally, as a sensitivity analysis, the fatty liver index (FLI) was utilized to validate the robustness of our results.

All data analyses were performed using R software (<https://www.r-project.org/>; version 4.2.1). A bilateral $P < 0.05$ was considered statistically different.

3 Results

3.1 Population characteristics

A total of 10,821 subjects were enrolled in this study (Supplementary Figure 1). Table 1 shows the demographic characteristics of all the participants. Among the participants, 38.48% fell within the age group of 40–59 years, and 51.00% were female. Notably, individuals with NAFLD exhibited higher household incomes, levels of education, and a higher prevalence of diabetes, hypertension, and hyperlipidemia compared to those without NAFLD. Figure 1 shows the proportion of patients with NAFLD sorted by quartiles of ln-transformed SII, NLR, PLR, and LMR. Higher quartiles of SII and NLR were associated with a higher prevalence of NAFLD, while conversely, higher quartiles of PLR showed a lower prevalence of NAFLD. In contrast, quartiles of LMR demonstrated similar proportions of NAFLD.

3.2 Association of SII, NLR, PLR, and LMR with NAFLD risk

Table 2 shows the relationship between SII, NLR, PLR, LMR, and risk of NAFLD. We constructed three models by adjusting for different confounding variables to evaluate the relationship between SII, NLR, PLR, LMR, and NAFLD risk. After adjusting for all confounding variables (model 2), there was a significant positive association observed between ln-transformed SII (OR= 1.46, 95% CI: 1.27–1.69, $P < 0.001$), NLR (OR= 1.25, 95% CI: 1.05–1.49, $P = 0.015$), LMR (OR= 1.39, 95% CI: 1.14–1.69, $P = 0.002$) with NAFLD prevalence. However, in the final model, the relationship between ln(PLR) (OR= 0.85, 95% CI: 0.70–1.03, $P = 0.092$) and

NAFLD risk was not significant. Consistent with this result, this trend was consistently observed when ln-transformed SII, NLR, PLR, and LMR were considered categorical variables (quartiles) in the sensitivity analysis. In the fully adjusted model (model 2), the risk of NAFLD increased progressively in the highest quartile group of SII, NLR, and LMR (Q4) compared with the lowest quartile group (Q1) (P for trend < 0.05). In addition, we observed that this trend also became meaningful when PLR was used as a quartile (P for trend < 0.05).

In parallel, we also analyzed the primary cell subpopulations for these cell ratios (Supplementary Table 1). After adjusting for all confounding variables (model 2), significant positive correlations were found between ln-transformed neutrophil count (OR= 2.62, 95% CI: 2.11–3.25, $P < 0.001$), platelet count (OR= 2.39, 95% CI: 1.88–3.04, $P < 0.001$), lymphocyte count (OR= 2.23, 95% CI: 1.72–2.88, $P < 0.001$), monocyte count (OR= 1.45, 95% CI: 1.18–1.77, $P < 0.001$) and NAFLD risk. The results remained unchanged when these cell subpopulations were used as categorizing variables.

3.3 Dose-response of systemic immune-inflammatory biomarkers (SII, NLR, PLR, and LMR) and NAFLD risk

To further ensure the robustness of the results, we investigated whether there was a nonlinear relationship between systemic immune-inflammatory biomarkers (SII, NLR, PLR, and LMR) and NAFLD risk. As shown in Figure 2, in the RCS regression model adjusting for all confounders, there was no nonlinear relationship between SII, NLR, LMR and NAFLD (P for nonlinearity > 0.05). This aligns with the linear regression outcomes described earlier. Interestingly, we observed an inverted “U”-shaped nonlinear dose-response relationship for PLR and the risk of NAFLD (P for nonlinearity < 0.05), prompting further investigation. Subsequently, in the segmented regression and threshold analysis (Table 3), the results showed an inflection point value of 4.64 for ln(PLR). When ln(PLR) was below 4.64, each unit increase in ln(PLR) was associated with a 0.55-fold increase in the risk of NAFLD (OR= 1.55, 95% CI: 1.05–2.31, $P < 0.05$). Conversely, when ln(PLR) exceeded 4.64, each unit increase in ln(PLR) was associated with a 0.40-fold decrease in the risk of NAFLD (OR= 0.60, 95% CI: 0.44–0.81, $P < 0.05$) (log-likelihood test: 0.001).

3.4 Subgroup analyses and sensitivity analyses

Figure 3 demonstrates the relationship between systemic immune-inflammatory biomarkers (SII, NLR, PLR, and LMR) and NAFLD risk within diabetic and non-diabetic subgroups. It was found that none of the interactions between ln-transformed systemic immune-inflammatory biomarkers (SII, NLR, PLR, and LMR) and diabetes were significant (all P for interaction > 0.05). Additionally, we conducted subgroup analyses for age, gender, race, PIR, education level, BMI, hypertension, and hyperlipidemia

TABLE 1 Characteristics of the study population.

Characteristic	Overall (n = 10821)	Non-NAFLD (n = 7496)	NAFLD (n = 3325)	P value
Age, n (%)				<0.001
20-39 years	3348.00 (34.81%)	2625.00 (38.67%)	723.00 (25.05%)	
40-59 years	3773.00 (38.48%)	2589.00 (38.03%)	1184.00 (39.62%)	
≥60 years	3700.00 (26.71%)	2282.00 (23.30%)	1418.00 (35.33%)	
Gender, n (%)				<0.001
Female	5522.00 (51.00%)	3964.00 (53.15%)	1558.00 (45.58%)	
Male	5299.00 (49.00%)	3532.00 (46.85%)	1767.00 (54.42%)	
Race/ethnicity, n (%)				<0.001
Mexican American	1622.00 (8.08%)	819.00 (6.14%)	803.00 (12.99%)	
Other Hispanic	1123.00 (5.36%)	737.00 (5.23%)	386.00 (5.70%)	
Non-Hispanic White	4801.00 (68.89%)	3291.00 (68.53%)	1510.00 (69.79%)	
Non-Hispanic Black	2048.00 (10.31%)	1694.00 (12.11%)	354.00 (5.76%)	
Other Race	1227.00 (7.36%)	955.00 (7.99%)	272.00 (5.76%)	
PIR, n (%)				<0.001
≤1.3	3343.00 (21.17%)	2187.00 (20.50%)	1156.00 (22.88%)	
1.3-3.5	4125.00 (36.22%)	2819.00 (34.93%)	1306.00 (39.47%)	
>3.5	3353.00 (42.61%)	2490.00 (44.57%)	863.00 (37.65%)	
Education level, n (%)				<0.001
Less than high school	2461.00 (14.99%)	1472.00 (13.21%)	989.00 (19.49%)	
High school or equivalent	2431.00 (22.45%)	1681.00 (21.88%)	750.00 (23.90%)	
Some college or more	5929.00 (62.56%)	4343.00 (64.91%)	1586.00 (56.61%)	
BMI, n (%)				<0.001
<25 kg/m ²	2977.00 (28.67%)	2845.00 (38.68%)	132.00 (3.41%)	
25-30 kg/m ²	3615.00 (33.12%)	2794.00 (37.55%)	821.00 (21.95%)	
≥30 kg/m ²	4229.00 (38.21%)	1857.00 (23.77%)	2372.00 (74.65%)	
Smoking status, n (%)	4823.00 (44.35%)	3284.00 (43.74%)	1539.00 (45.88%)	0.113
Diabetes, n (%)				<0.001
No	8668.00 (85.27%)	6590.00 (91.89%)	2078.00 (68.55%)	
Yes	2153.00 (14.73%)	906.00 (8.11%)	1247.00 (31.45%)	
Hypertension, n (%)				<0.001
No	6048.00 (60.85%)	4653.00 (67.19%)	1395.00 (44.84%)	
Yes	4773.00 (39.15%)	2843.00 (32.81%)	1930.00 (55.16%)	
Hyperlipidemia, n (%)				<0.001
No	2573.00 (25.37%)	2199.00 (30.76%)	374.00 (11.77%)	
Yes	8248.00 (74.63%)	5297.00 (69.24%)	2951.00 (88.23%)	
ALT (U/L)	25.24 (17.21)	22.97 (15.25)	30.95 (20.29)	<0.001
AST (U/L)	24.96 (16.72)	24.35 (16.44)	26.51 (17.32)	<0.001
Neutrophil count (1000 cell/μL)	3.95 (1.60)	3.75 (1.55)	4.46 (1.60)	<0.001

(Continued)

TABLE 1 Continued

Characteristic	Overall (n = 10821)	Non-NAFLD (n = 7496)	NAFLD (n = 3325)	P value
Platelet count (1000 cell/ μ L)	239.92 (61.89)	237.22 (60.23)	246.73 (65.42)	<0.001
Lymphocyte count (1000 cell/ μ L)	2.01 (0.96)	1.96 (1.01)	2.14 (0.83)	<0.001
Monocyte count (1000 cell/ μ L)	0.54 (0.20)	0.52 (0.17)	0.58 (0.24)	<0.001
SII	512.30 (325.17)	493.93 (328.08)	558.65 (313.00)	<0.001
NLR	2.13 (1.09)	2.08 (1.06)	2.27 (1.14)	<0.001
PLR	129.77 (48.82)	131.84 (50.07)	124.55 (45.11)	<0.001
LMR	4.00 (1.58)	3.99 (1.55)	4.02 (1.65)	0.417

PIR, family poverty income ratio; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; SII, systemic immune-inflammation index; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio.

(Table 4). Consistently, the majority of subgroup analyses reaffirmed the lack of significant interactions (P for interaction >0.05). Furthermore, when ln-transformed SII, NLR, PLR, and LMR were considered as categorical variables (quartiles), we again performed subgroup analyses, and the results of all subgroup analyses similarly confirmed this finding (Supplementary Tables 2-5).

In the sensitivity analysis, NAFLD was defined using an FLI score ≥ 60 . The results (Supplementary Table 6) showed that in the fully adjusted model, ln-transformed SII (OR= 1.40, 95% CI: 1.24-1.58, P <0.001), NLR (OR= 1.18, 95% CI: 1.03-1.36, P =0.021), LMR (OR= 1.54, 95% CI: 1.26-1.88, P <0.001) remained significantly positively correlated with the prevalence of NAFLD. The association between ln(PLR) (OR= 0.92, 95% CI: 0.76-1.11, P =0.372) and risk of NAFLD remained non-significant (P >0.05). This result still supports our prior findings when ln-transformed SII, NLR, PLR, and LMR are considered categorical variables (quartiles), which indicates the robustness of our results.

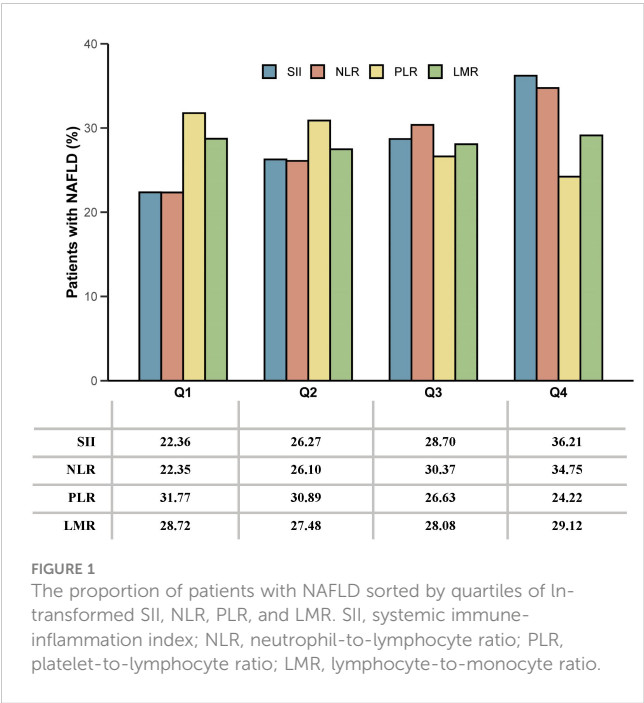


FIGURE 1
The proportion of patients with NAFLD sorted by quartiles of ln-transformed SII, NLR, PLR, and LMR. SII, systemic immune-inflammation index; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio.

4 Discussion

The association between systemic immune-inflammatory biomarkers and NAFLD in the American population was exhaustively examined by our research. We normalized the systemic inflammatory indices using ln-transformation. The results revealed a statistically significant positive correlation between SII, NLR, LMR, and NAFLD, underscoring that elevated values of SII, NLR, and LMR were linked to an increased risk of NAFLD. Furthermore, a nonlinear dose-response relationship was observed for PLR, characterized by an inverted “U”-shape. The nadir of NAFLD risk occurred at ln(PLR) 4.64, with a positive association observed below this threshold and a negative association above it. Further subgroup analyses showed that the associations of SII, NLR, PLR, and LMR between diabetic and nondiabetic populations with NAFLD were not significantly different, and this association was similar in other different subgroups. In conclusion, our findings suggest that SII, NLR, PLR, and LMR are strongly associated with NAFLD risk and emphasize the robustness of the findings.

The development of NAFLD is closely associated with metabolic and inflammatory disorders (35). However, the conclusions of previous relevant studies were controversial. Zhao et al. found that SII of NAFLD was non-linear associated with all-cause mortality and that an elevated SII was positively associated with reduced survival in patients with NAFLD (36). Some studies have also shown the SII was linked with NAFLD risk in a “U”-shaped pattern, and subgroup analyses showed a positive association between the SII index and the risk of NAFLD in participants without diabetes (37). Another cross-sectional study showed a nonlinear association between NLR and PLR and NAFLD, with $PLR \geq 42.29$ as a protective factor of NAFLD, and $NLR < 1.23$ might be a risk factor of NAFLD (20). Clinical studies have shown that patients with NAFLD have higher NLR and LMR were higher than healthy controls (P <0.001), while PLR was significantly lower (38, 39). Our findings are in general agreement with the literature supporting the association of elevated systemic immune-inflammatory biomarkers with increased risk of NAFLD, demonstrating the important role of inflammation in the pathogenesis of NAFLD.

TABLE 2 The relationship between SII, NLR, PLR, and LMR and the risk of NAFLD.

Characteristic	Crude model ^a		Model 1 ^b		Model 2 ^c	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
SII (ln-transformed)	1.66 (1.48, 1.85)	<0.001	1.61 (1.43, 1.82)	<0.001	1.46 (1.27, 1.69)	<0.001
SII (Quartile)						
Q1	Ref		Ref		Ref	
Q2	1.24 (1.09, 1.40)	<0.001	1.20 (1.06, 1.36)	0.006	1.15 (0.96, 1.37)	0.118
Q3	1.40 (1.21, 1.62)	<0.001	1.35 (1.16, 1.57)	<0.001	1.19 (0.98, 1.45)	0.074
Q4	1.97 (1.73, 2.25)	<0.001	1.91 (1.66, 2.20)	<0.001	1.69 (1.39, 2.05)	<0.001
P for trend		<0.001		<0.001		<0.001
NLR (ln-transformed)	1.63 (1.42, 1.88)	<0.001	1.39 (1.21, 1.61)	<0.001	1.25 (1.05, 1.49)	0.015
NLR (Quartile)						
Q1	Ref		Ref		Ref	
Q2	1.23 (1.06, 1.42)	0.007	1.13 (0.97, 1.32)	0.123	1.07 (0.87, 1.31)	0.539
Q3	1.51 (1.29, 1.77)	<0.001	1.38 (1.17, 1.63)	<0.001	1.17 (0.95, 1.43)	0.132
Q4	1.85 (1.59, 2.16)	<0.001	1.56 (1.32, 1.84)	<0.001	1.36 (1.08, 1.72)	0.010
P for trend		<0.001		<0.001		0.007
PLR (ln-transformed)	0.68 (0.58, 0.79)	<0.001	0.63 (0.54, 0.75)	<0.001	0.85 (0.70, 1.03)	0.092
PLR (Quartile)						
Q1	Ref		Ref		Ref	
Q2	0.96 (0.83, 1.10)	0.564	0.95 (0.83, 1.10)	0.526	1.14 (0.94, 1.37)	0.179
Q3	0.78 (0.66, 0.91)	0.003	0.76 (0.63, 0.90)	0.002	0.88 (0.69, 1.12)	0.294
Q4	0.69 (0.58, 0.81)	<0.001	0.65 (0.55, 0.76)	<0.001	0.85 (0.70, 1.03)	0.100
P for trend		<0.001		<0.001		0.033
LMR (ln-transformed)	1.00 (0.87, 1.14)	0.996	1.45 (1.24, 1.70)	<0.001	1.39 (1.14, 1.69)	0.002
LMR (Quartile)						
Q1	Ref		Ref		Ref	
Q2	0.94 (0.80, 1.11)	0.458	1.10 (0.93, 1.30)	0.247	1.10 (0.89, 1.37)	0.364
Q3	0.97 (0.83, 1.14)	0.699	1.22 (1.02, 1.45)	0.026	1.21 (0.98, 1.50)	0.074
Q4	1.02 (0.89, 1.17)	0.784	1.45 (1.24, 1.69)	<0.001	1.39 (1.16, 1.67)	<0.001
P for trend		0.701		<0.001		0.001

OR, odds ratio; CI, confidence interval; Q, quartile; SII, systemic immune-inflammation index; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio.

^aThe crude model was not adjusted for any covariates.

^bModel 1 was adjusted for age, gender, and race.

^cModel 2 was adjusted for all covariates based on model 1.

In the 2010s, 20% to 30% of the U.S. population met the criteria for NAFLD and the prevalence continues to increase, with NAFLD and NASH more prevalent in men (3, 40, 41). In contrast, our baseline results showed a similar proportion of men and women, with no significant differences seen. The largest proportion of people were aged 40-59 years in our study. A cohort study showed that risk factors, prevalence, and characteristics of NAFLD patients varied by age group (42). Consider this about the fact that senescent cells cause age-related tissue degeneration and that the accumulation of senescent cells promotes hepatic fat accumulation and steatosis. Senescence-associated mitochondrial dysfunction reduces cellular fatty acid oxidation capacity resulting in increased fat deposition capacity resulting in increased fat deposition (43). In addition, NAFLD is strongly associated with obesity, dyslipidemia, type 2 diabetes mellitus, and metabolic syndrome, which is consistent with our baseline characteristics, and patients with NAFLD have higher rates of developing diabetes mellitus, hypertension, hyperlipidemia, and higher BMI values. It

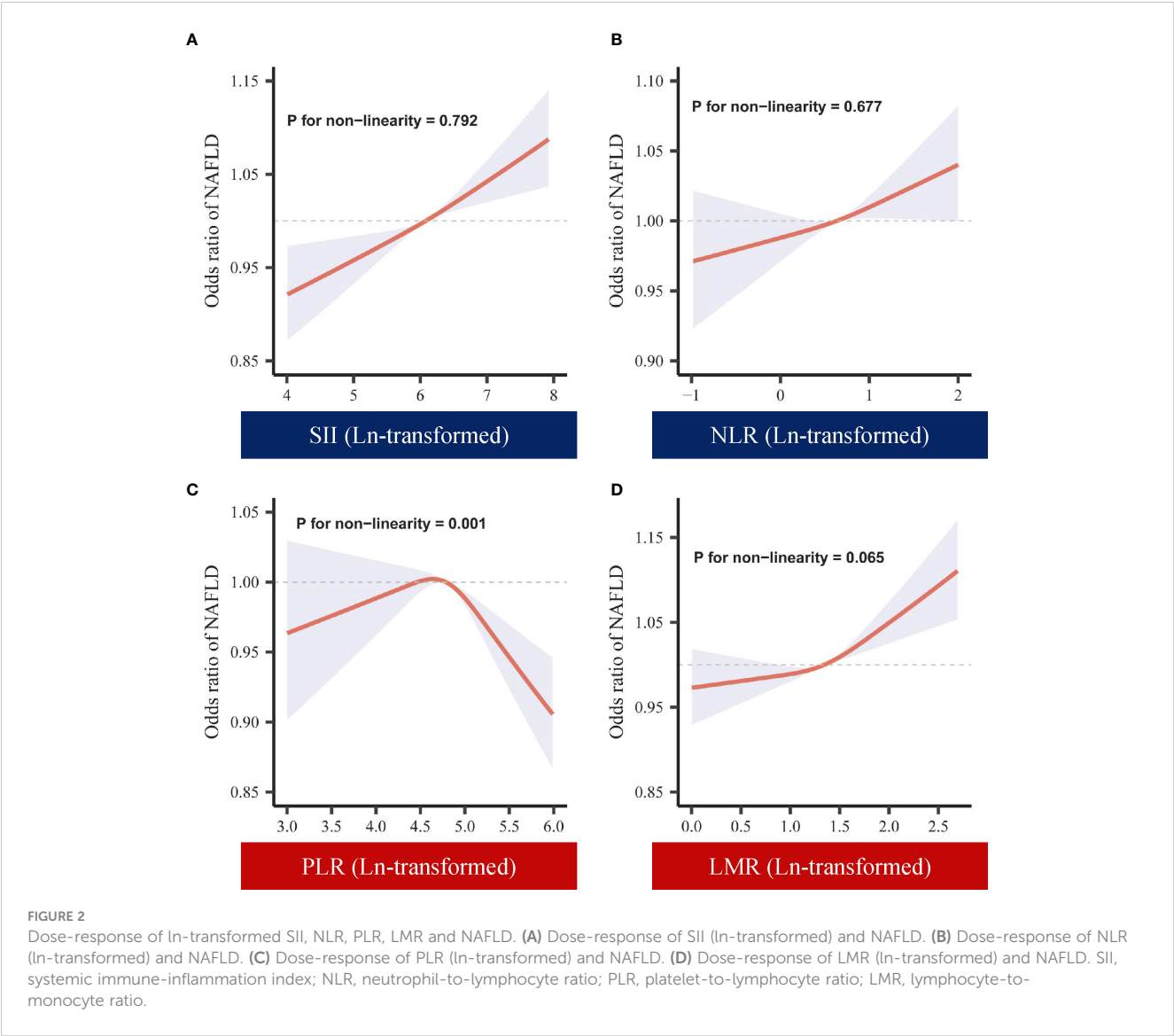


TABLE 3 The threshold effect of PLR (ln-transformed) on NAFLD was analyzed using a two-stage phased regression model.

Models	Adjusted OR (95% CI) ^a	P value
Model I		
logistic regression (the standard linear model)	0.85 (0.70, 1.03)	0.092
Model II		
Inflection point	4.64	
<4.64	1.55 (1.05, 2.31)	0.029
>4.64	0.60 (0.44, 0.81)	0.001
Log likelihood ratio ^b		0.001

OR, Odds Ratio; CI, Confidence Interval; PLR, platelet-to-lymphocyte ratio.
^aAdjusted for age, gender, race, family poverty income ratio, education level, smoking status, body mass index, diabetes, hypertension, hyperlipidemia, and alanine aminotransferase.
^bModel II compared to model I.

may be related to the impairment of amino acid metabolism in NAFLD, and insulin resistance, which leads to the accumulation of fat in the liver, which in turn leads to a greater influx of free fatty acids into the liver, and the accumulation of fat in the liver leads to an inflammatory response (44).

Inflammatory immune response plays a key role in the development of NAFLD. In NAFLD, pro-death and other programmed deaths caused by classical or non-classical inflammasome pathways play an important role in promoting, and some cellular components released after cell death can cause a strong inflammatory response and promote the recruitment of inflammatory cells (45–47). This is consistent with our findings that elevated SII is positively associated with an increased risk of NAFLD. However, the specific mechanism needs further study. In the “two-hit hypothesis” for the progression of NAFLD, a “first hit” occurs due to liver fat accumulation and insulin resistance, resulting in a reduced sensitivity of the liver to further inflammation, leading to the development of NASH. This is associated with increased NLR and insulin resistance. Its development involves the death of

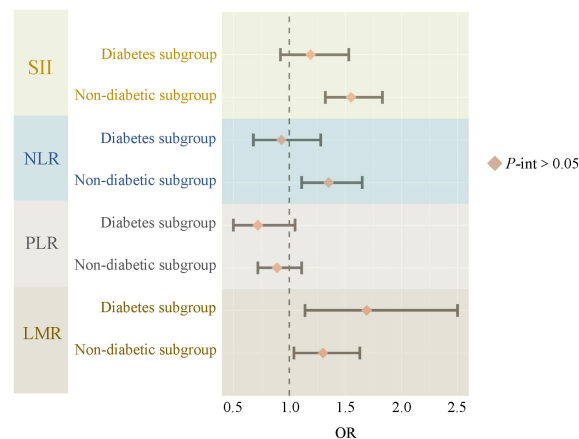


FIGURE 3

The relationship between SII, NLR, PLR, LMR and NAFLD risk in diabetes subgroups. SII, systemic immune-inflammation index; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; P-int, P for interaction.

hepatocytes through apoptosis and necrosis, which in turn activates macrophages, neutrophils, and pro-inflammatory pathways. The “second hit” then involves the activation of systemic pro-inflammatory pathways, particularly the increase in inflammatory cytokines, chemokines, and signaling molecules. Among them, nuclear factor-kappa B (NF- κ B) and c-Jun N-terminal kinase (JNK) as the key pro-inflammatory signal molecules increased in NASH, as these signaling pathways provide a link between hepatic inflammation and insulin resistance (48). There is evidence that fat accumulation causes inflammation in the liver, activating Kupffer cells and releasing inflammatory cytokines, which enter the bloodstream and trigger a systemic inflammatory response. Qi et al. found that CXCL5 increased the lipid toxicity of hepatocytes by up-regulating NLRP3/caspase1/IL-1 β signaling in KCs and exerting its pro-inflammatory properties (49). Liu et al. confirmed that CARD9 deficiency induces S100a8/a9 expression through toll-like receptors, leading to increased expression of pro-inflammatory, fibrotic, and lipid metabolism-related genes in NASH progression (50). In addition, in NAFLD, platelets are highly activated and participate in disease progression by enhancing the pro-thrombotic and pro-inflammatory states. Platelets can cause sinusoidal endothelial cells to release a large number of chemokines, increase the migration of neutrophils and lymphocytes, and induce liver injury. On the other hand, platelets can cause liver inflammation by enhancing the recruitment of white blood cells in the sinusoidal endothelium, and can further activate effector cells, thereby amplifying liver injury. Various long-term studies have shown that platelets induce the progression of NAFLD primarily by generating a pro-inflammatory and pro-fibrotic environment in the liver (51, 52). In the presence of inflammatory diseases, circulating lymphocytes are often reduced (48). The “U”-shaped nonlinear relationship between PLR and NAFLD in our study also suggests that there is a certain correlation between them. In general, the relationship between systemic immune-inflammatory biomarkers and NAFLD needs to be further studied. The significant association between systemic immune-inflammatory biomarkers and NAFLD, as well as previous literature, still indicates that systemic immune-

inflammatory biomarkers participate in or may affect the occurrence of NAFLD. In any case, we know that this is still speculative and that further evidence is needed to clarify its causal relationship and mechanism of action to better make it a predictor of NAFLD risk.

Compared to earlier studies, our paper has these points. First, fewer previous studies have observed an association between systemic immune-inflammatory biomarkers and NAFLD and the results have been inconsistent (25, 36, 37, 53, 54). Our study provides new information on the quantitative relationship between the systemic immune-inflammatory biomarkers (SII, NLR, PLR, and LMR) and NAFLD indicators in the general U.S. population and explores the risk relationship between the two. Second, in contrast to previous studies, we evaluated the association between NAFLD and inflammatory markers obtained from complete blood counts (CBCs), including SII, NLR, PLR, and LMR, which is one of the most common tests used in clinical work. However, these four items have not been comprehensively addressed in previous studies and are usually analyzed as single indicators. This time, the indexes were included more comprehensively in our study, and the correlation between multiple indices and NAFLD was analyzed more specifically.

Our study also has some limitations. First, due to the cross-sectional design of NHANES, we were unable to determine a causal relationship between systemic immune-inflammatory biomarkers and NAFLD. Second, considering the potential enduring impact of inflammation on NAFLD, the utilization of anti-inflammatory drugs in certain patients prompts a need for cautious interpretation of the results. Consequently, there is an imperative for more prospective studies with larger sample sizes to further elucidate and validate these findings. In addition, the current findings may not be compelling for other racial groups because non-Hispanic whites are more predominant in NHANES. Different racial backgrounds may exhibit different prevalence rates, and these data sets may lead to regional bias. Finally, despite our meticulous consideration of multiple covariates for adjustment, the specter of residual confounding looms. The potential impact of unmeasured

TABLE 4 The relationship between SII, NLR, PLR, LMR and NAFLD risk in different subgroups.

Characteristic	SII (ln-transformed)		NLR (ln-transformed)		PLR (ln-transformed)		LMR (ln-transformed)	
	OR (95% CI)	<i>P</i> -int	OR (95% CI)	<i>P</i> -int	OR (95% CI)	<i>P</i> -int	OR (95% CI)	<i>P</i> -int
Age, n (%)		0.028		0.493		0.337		0.522
20-39 years	1.76 (1.37, 2.25)		1.39 (1.02, 1.89)		1.05 (0.70, 1.58)		1.47 (1.03, 2.12)	
40-59 years	1.64 (1.25, 2.14)		1.35 (0.97, 1.89)		0.81 (0.57, 1.14)		1.40 (0.98, 1.99)	
≥60 years	1.12 (0.91, 1.38)		1.04 (0.82, 1.32)		0.75 (0.56, 1.00)		1.35 (1.02, 1.79)	
Gender, n (%)		0.464		0.341		0.058		0.192
Male	1.37 (1.15, 1.63)		1.13 (0.94, 1.37)		0.92 (0.73, 1.16)		1.33 (1.06, 1.67)	
Female	1.59 (1.29, 1.96)		1.41 (1.07, 1.87)		0.79 (0.60, 1.03)		1.42 (1.03, 1.94)	
Race/ethnicity, n (%)		0.043		0.136		0.126		0.294
Mexican American	1.15 (0.90, 1.48)		1.02 (0.77, 1.35)		0.75 (0.52, 1.09)		1.51 (1.06, 2.14)	
Other Hispanic	1.21 (0.84, 1.76)		1.23 (0.81, 1.89)		0.61 (0.35, 1.07)		1.04 (0.63, 1.74)	
Non-Hispanic White	1.43 (1.16, 1.75)		1.18 (0.91, 1.51)		0.82 (0.64, 1.05)		1.49 (1.13, 1.97)	
Non-Hispanic Black	1.46 (1.16, 1.84)		1.46 (1.11, 1.92)		0.87 (0.63, 1.21)		1.29 (0.84, 1.96)	
Other Race	2.96 (1.88, 4.66)		2.52 (1.43, 4.46)		1.84 (1.02, 3.31)		0.83 (0.46, 1.49)	
PIR, n (%)		0.910		0.906		0.553		0.042
≤1.3	1.47 (1.18, 1.83)		1.23 (0.93, 1.61)		0.99 (0.74, 1.31)		1.87 (1.38, 2.55)	
1.3-3.5	1.45 (1.20, 1.75)		1.27 (0.97, 1.65)		0.83 (0.62, 1.13)		1.27 (0.94, 1.73)	
>3.5	1.46 (1.11, 1.92)		1.22 (0.89, 1.68)		0.78 (0.54, 1.13)		1.26 (0.87, 1.83)	
Education level, n (%)		0.362		0.217		0.283		0.194
Less than high school	1.57 (1.24, 2.00)		1.47 (1.11, 1.95)		0.98 (0.67, 1.45)		1.33 (0.90, 1.96)	
High school or equivalent	1.45 (1.11, 1.91)		1.33 (0.97, 1.84)		0.91 (0.62, 1.34)		1.76 (1.19, 2.61)	
Some college or more	1.43 (1.17, 1.75)		1.16 (0.92, 1.47)		0.80 (0.62, 1.05)		1.25 (0.95, 1.63)	
Smoking status, n (%)		0.870		0.597		0.222		0.812
Yes	1.40 (1.16, 1.70)		1.22 (1.00, 1.49)		0.88 (0.68, 1.13)		1.40 (1.06, 1.83)	
No	1.50 (1.23, 1.82)		1.26 (0.98, 1.60)		0.81 (0.63, 1.05)		1.37 (1.05, 1.78)	
BMI, n (%)		0.659		0.813		0.903		0.104
<25 kg/m ²	1.14 (0.70, 1.87)		0.98 (0.54, 1.79)		0.83 (0.43, 1.59)		1.01 (0.55, 1.86)	
25-30 kg/m ²	1.33 (1.07, 1.65)		1.11 (0.88, 1.40)		0.90 (0.67, 1.21)		1.49 (1.07, 2.07)	
≥30 kg/m ²	1.53 (1.26, 1.86)		1.32 (1.04, 1.69)		0.81 (0.64, 1.03)		1.38 (1.09, 1.74)	
Hypertension, n (%)		0.565		0.777		0.423		0.985
Yes	1.38 (1.13, 1.67)		1.26 (0.99, 1.60)		0.88 (0.71, 1.09)		1.36 (1.06, 1.74)	
No	1.55 (1.26, 1.91)		1.20 (0.92, 1.58)		0.79 (0.57, 1.08)		1.41 (1.05, 1.90)	
Diabetes, n (%)		0.172		0.050		0.662		0.176
Yes	1.19 (0.92, 1.53)		0.93 (0.68, 1.28)		0.72 (0.50, 1.05)		1.69 (1.14, 2.50)	
No	1.55 (1.32, 1.83)		1.35 (1.11, 1.65)		0.89 (0.72, 1.11)		1.30 (1.04, 1.63)	
Hyperlipidemia, n (%)		0.259		0.228		0.555		0.223
Yes	1.42 (1.21, 1.66)		1.20 (1.00, 1.44)		0.84 (0.69, 1.02)		1.45 (1.18, 1.78)	
No	1.65 (1.19, 2.29)		1.43 (0.91, 2.25)		0.89 (0.55, 1.46)		1.06 (0.55, 2.05)	

OR, odds ratio; CI, confidence interval; P-int, P for interaction; SII, systemic immune-inflammation index; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PIR, family poverty income ratio; BMI, body mass index.

factors on our results cannot be entirely discounted, and there exists a risk of bias to some degree due to residual confounding.

5 Conclusions

In summary, findings derived from a cohort of U.S. adults indicate a noteworthy association between systemic immune-inflammatory biomarkers (SII, NLR, PLR, and LMR) and the risk of NAFLD. Specifically, elevated SII, NLR, and LMR were identified as significant contributors to an increased risk of NAFLD. Notably, the relationship between PLR and NAFLD exhibited a nonlinear pattern, suggesting a nuanced impact that is not strictly monotonic. Therefore, we emphasized the role of systemic immune-inflammatory biomarkers for NAFLD risk prediction. However, in the future, further exploration of the causal relationship between these systemic immune-inflammatory biomarkers and NAFLD is warranted.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: <https://www.cdc.gov/nchs/nhanes>.

Ethics statement

The studies involving humans were approved by the Centers for Disease Control and Prevention (CDC) and the National Center for Health Statistics (NCHS). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

KL: Methodology, Writing – original draft, Writing – review & editing. ST: Methodology, Writing – original draft, Writing – review & editing. CL: Methodology, Writing – original draft, Writing – review & editing. JM: Data curation, Formal analysis, Writing – review & editing. XC: Data curation, Formal analysis, Writing – review & editing. XY: Data curation, Writing – review & editing. YZ: Data curation, Writing – review & editing. KC: Data curation, Writing – review & editing. YaL: Funding acquisition, Resources, Supervision, Writing – original draft, Writing – review & editing. CZ: Funding acquisition, Resources, Supervision, Writing – original draft, Writing – review & editing. YiL: Funding acquisition,

Resources, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fimmu.2024.1337241/full#supplementary-material>

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Interferons and interferon-related pathways in heart disease

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Interferons (IFNs) and IFN-related pathways play key roles in the defence against microbial infection. However, these processes may also be activated during the pathogenesis of non-infectious diseases, where they may contribute to organ injury, or function in a compensatory manner. In this review, we explore the roles of IFNs and IFN-related pathways in heart disease. We consider the cardiac effects of type I IFNs and IFN-stimulated genes (ISGs); the emerging role of the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway; the seemingly paradoxical effects of the type II IFN, IFN- γ ; and the varied actions of the interferon regulatory factor (IRF) family of transcription factors. Recombinant IFNs and small molecule inhibitors of mediators of IFN receptor signaling are already employed in the clinic for the treatment of some autoimmune diseases, infections, and cancers. There has also been renewed interest in IFNs and IFN-related pathways because of their involvement in SARS-CoV-2 infection, and because of the relatively recent emergence of cGAS-STING as a pattern recognition receptor-activated pathway. Whether these advances will ultimately result in improvements in the care of those experiencing heart disease remains to be determined.

KEYWORDS

interferon, interferon-stimulated gene, heart failure, myocardial infarction, cGAS-STING, interferon regulatory factor, type I interferon (IFN), interferon gamma (IFN- γ)

Introduction

It is over sixty five years since a substance that interferes with viral replication in host cells, termed “interferon”, was first reported by Isaacs and Lindemann (1). Since that first description, our knowledge of interferons (IFNs), their upstream regulators, downstream effects, and related regulatory factors continues to expand well beyond the field of virology. IFNs and IFN-related pathways are emerging as critical determinants of the pathogenesis of heart disease, or indeed on occasion protection against it. In this review article, we discuss the emerging state-of-the-art of IFNs and IFN-related pathways in the heart focusing on type I IFNs, the type I IFN response, and interferon-stimulated genes (ISGs); the related upstream cyclic GMP-AMP synthase (cGAS)- stimulator of interferon genes (STING) signaling pathway; the seemingly paradoxical actions of the type II IFN, IFN- γ ; and the interferon regulatory factor (IRF) family of transcription factors.

The IFN family, IFN function and IFN induction

IFNs belong to the Class II cytokine family, a group of α -helical cytokines with modest sequence homology but structural similarity (2). The IFN family itself is made up of three classes, distinguished from one another according to the type of receptor that they bind to: type I IFNs, type II IFN (of which there is only one), and type III IFNs (Figure 1). The type

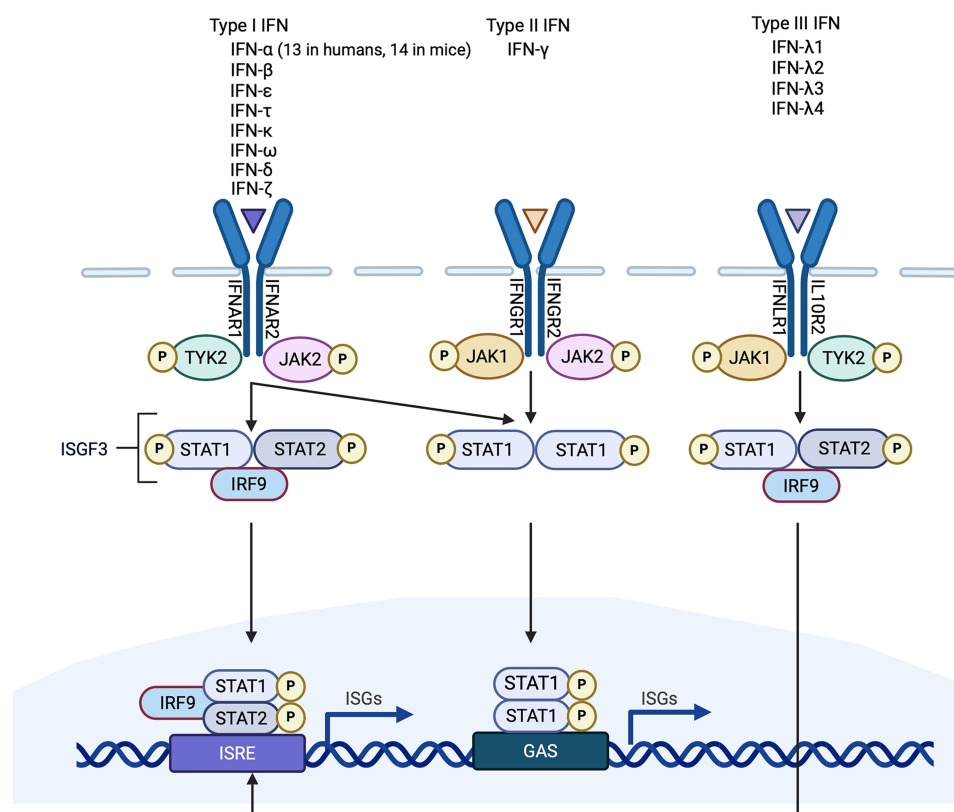


FIGURE 1

Simplified schematic of interferon (IFN) signaling pathways. Type I IFNs are the largest of the three classes of IFN. They signal through IFNAR1/2 which activates JAK/STAT signaling. STAT1/2 phosphorylation causes the release of STATs from IFNAR and the formation of a complex containing phosphorylated STAT1/2 and IRF9, called interferon-stimulated gene factor 3 (ISGF3). ISGF3 binds to a promoter sequence called interferon-stimulated response element (ISRE) and induces interferon stimulated genes (ISGs). There is only one Type II IFN (IFN- γ). It binds to IFNGR1/2 to activate JAK/STAT signaling and the formation of phosphorylated STAT1 homodimers which bind to IFN- γ -activated sites (GAS) elements in gene promoters. Type I IFNs can also induce STAT1 homodimerization. Type III IFNs signal through IFNLR1/IL10R2 and, like type I IFNs, induce the formation of an ISGF3 complex. Non-canonical signaling pathways are not shown.

I IFNs consist of five α -helices (3), and bind to a ubiquitously expressed heterodimeric receptor that is made of two chains called IFNAR1 and IFNAR2. Within the type I IFN class, and the most extensively studied of the IFNs, are IFN- α and IFN- β . Thirteen genes encode for human IFN- α (in mice there are 14), whereas IFN- β is encoded by a single gene (3, 4). Other type I IFNs are less well characterized. These include IFN- ϵ , IFN- τ , IFN- κ , IFN- ω , IFN- δ , and IFN- ξ (3). There is only one member of the type II IFN class, IFN- γ . Whereas type I IFNs are monomers, IFN- γ is an intercalated dimer (3). IFN- γ is structurally unrelated to the type I IFNs and it binds to a different receptor, which is made up of IFNGR1 and IFNGR2 subunits (5). The type III IFNs [made up of IFN- λ 1, IFN- λ 2 and IFN- λ 3, also called interleukin-28A (IL-28A), IL-28B and IL-29 respectively, and IFN- λ 4] are structurally related to type I IFNs and also to IL-10 (5). They bind to a heterodimeric receptor made up of IL10R2 and IFNLR1 subunits (5), and they are the least characterized IFN class.

Almost all cells can be induced to express type I IFNs, although the main sources of type I IFNs are innate immune cells (6). IFN- γ

expression, in contrast, is more restricted, being primarily expressed by T cells [CD8+ cytotoxic T cells and T helper type 1 (Th1) cells] and natural killer (NK) cells (5). However, most cells express IFNGR and therefore most cells respond to IFN- γ (7). IFN- γ can induce the expression of genes that prime the type I IFN response, and type I IFNs can potentiate IFN- γ signaling (7, 8). Type III IFNs, like type I IFNs, can also be expressed by most cells, although they mainly act at epithelial surfaces (5, 9, 10).

The primary function of the IFNs is in the host defence against microbial infection. IFN gene expression is induced by the binding of pattern recognition receptors (PRRs) to pathogen-associated molecular patterns (PAMPs), which are molecules unique to microbes such as viruses or bacteria [e.g., nucleic acids, bacterial endotoxin (lipopolysaccharide, LPS), certain glycoproteins, bacterial peptides, and fungal glucans] (Figure 2). PRRs, however, can also be activated by endogenous molecules that are released by damaged or dying host cells. These endogenous molecules are called damage-associated molecular patterns (DAMPs). Outside of the setting of viral myocarditis or other microbial infections, induction of the IFN response in heart disease is mediated by

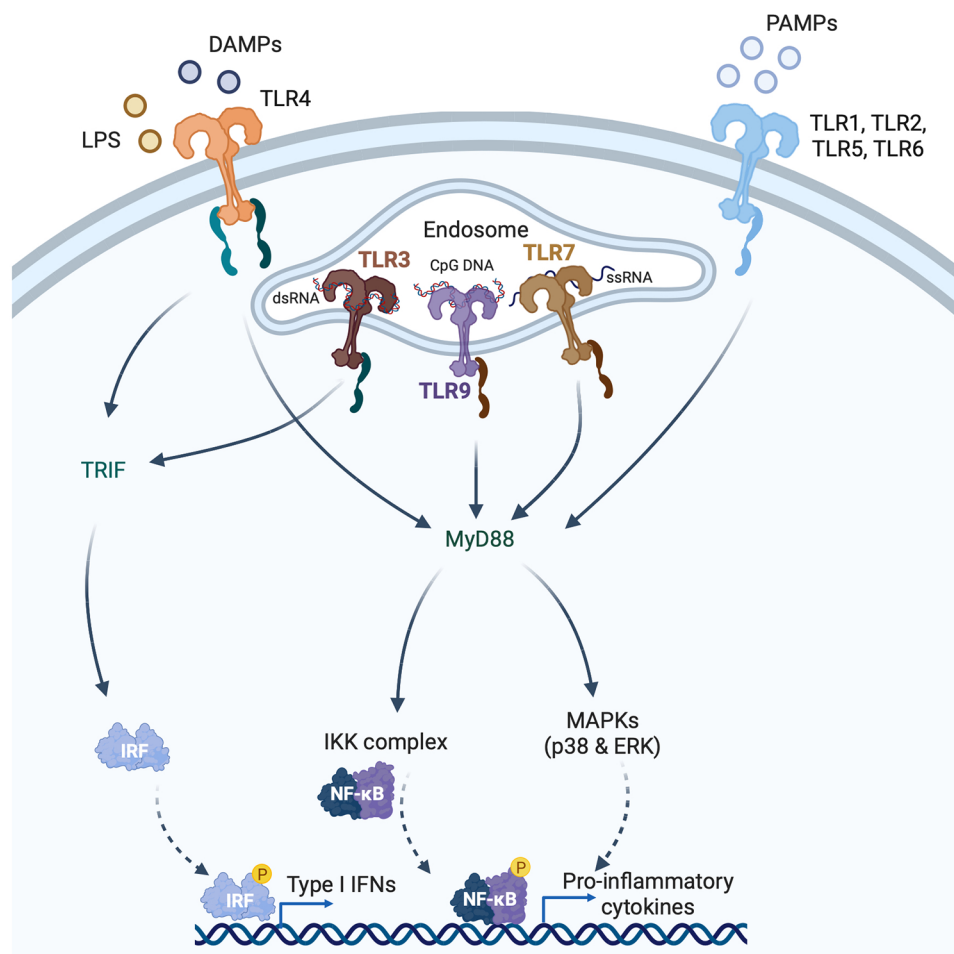


FIGURE 2

Induction of type I interferons (IFNs) by pattern recognition receptors (PRRs). PRRs recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) that are either intracellular or extracellular. Toll like receptors (TLRs) are membrane bound PRRs and major inducers of the IFN response. TLR4 is expressed on the cell surface and recognizes bacterial lipopolysaccharide (LPS) and endogenous DAMPs. TLR1, 2, 5 and 6 recognize PAMPs. TLR3 is localized to endosomes, and recognizes viral double-stranded RNA (dsRNA), whereas TLR7 recognizes single-stranded RNA, and TLR9 recognizes CpG DNA. TLRs induce signaling via either the myeloid differentiation primary response 88 (MyD88)-dependent or TIR domain-containing adaptor inducing IFN β (TRIF)-dependent pathways. TLR3 and TLR4 signal through the TRIF-dependent pathway, and TLR4 can also signal through the MyD88-dependent pathway. MyD88-signaling involves mitogen-activated protein kinase (MAPKs) and nuclear factor κ -light chain enhancer of activated B cells (NF κ B), whereas TRIF-signaling is mediated through IRF3 phosphorylation.

DAMPs. PRRs can sense PAMPs (or DAMPs) that are either outside the cell (through membrane bound PRRs) or inside the cell (through cytoplasmic PRRs). Membrane bound PRRs include Toll like receptors (TLRs) and C-type lectin receptors (CLRs). TLRs are major inducers of the IFN response. TLRs can be present on the cell membrane (mediating extracellular signaling) or on the membrane of endosomes (mediating intracellular signaling) (Figure 2). For instance, TLR3 is localized to endosomes, and recognizes viral double-stranded RNA (dsRNA), small interfering RNAs (siRNAs) and host RNAs derived from damaged cells (11). TLR4, on the other hand, is expressed on the cell surface and recognizes LPS derived from invading bacteria, and DAMPs produced by dying cells (5) (Figure 2). TLR7 recognizes single-stranded RNA (ssRNA). Its encoding gene is located on the X chromosome and frequently avoids X chromosome inactivation, which may be responsible for sex

differences in type I IFN responses (12). TLR9 is an intracellular TLR expressed on endosomes and the endoplasmic reticulum that senses DNA, especially unmethylated CpG DNA, which is more common in viruses and bacteria. After ligand-binding by TLRs, signal transduction is mediated by either the myeloid differentiation primary response 88 (MyD88)-dependent or TIR domain-containing adaptor inducing IFN- β (TRIF)-dependent pathways (11) (Figure 2). TLR3 and TLR4 signal through the TRIF-dependent pathway, and TLR4 can also signal through the MyD88-dependent pathway (11). Signaling by TLR7 and TLR9 is also MyD88 dependent. The MyD88-dependent pathway involves activation of mitogen-activated protein kinase (MAPK) and nuclear factor κ -light chain enhancer of activated B cells (NF κ B), whereas TRIF-dependent signaling is mediated through phosphorylation of IRF3. Both pathways ultimately result in the induction of proinflammatory genes, including IFNs (11).

Cytosolic PRRs recognize nucleic acids, such as viral nucleic acids, but also nucleic acids arising from damaged host cells or damaged mitochondria. For instance, RIG-I and melanoma differentiation-associated gene 5 (MDA5) sense viral dsRNA in the cytosol (13, 14), relaying signals via the adaptor protein mitochondrial antiviral sensing protein (MAVS) (15). Double-stranded DNA (dsDNA) aberrantly present in the cytosol can arise from invading microbes or from the host, including through leakage of mitochondrial DNA (mtDNA) from damaged mitochondria. Cytosolic dsDNA is sensed by cGAS, which relays signals via the adaptor protein, STING (16, 17). There has been substantial recent interest in the role that cGAS-STING pathway activation plays in organ injury, and the literature describing the contributions of cGAS-STING to heart disease is reviewed in the relevant section later in this review.

IFN signaling, IRFs and ISGs

All IFNs signal through the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway (Figure 1). Briefly, the intracellular domains of IFNAR1 and IFNAR2 are associated with two JAKs called non-receptor tyrosine-protein kinase 2 (TYK2) and JAK1 respectively. Ligand binding of IFNAR results in phosphorylation of the JAKs, which in turn phosphorylate tyrosine residues in the intracellular domains of the receptor subunits, as well as the STATs, STAT1 and STAT2 (18). STAT1/2 phosphorylation causes the release of the STATs from the IFNAR receptor and the formation of a trimeric complex that is comprised of STAT1, STAT2 and IRF9. This trimeric complex is called interferon-stimulated gene (ISG) factor 3, or ISGF3. ISGF3 translocates to the nucleus and acts as a transcriptional activator by binding to an interferon-stimulated response element (ISRE) in the promoter region of ISGs (5). Other type I IFN signaling pathways include the formation of STAT homodimers and heterodimers which can initiate gene transcription by binding to IFN- γ -activated site (GAS) elements in gene promoters (19). It has also been reported that IFNAR signaling can be mediated by the MAPK/c-Jun amino-terminal kinase (JNK) signaling pathway (8). Type II IFN signaling is also primarily JAK/STAT-mediated. IFN- γ binding of IFNGR1 and IFNGR2 induces association and phosphorylation of the receptor subunits with JAK1 and JAK2 respectively (5) (Figure 1). This results in STAT1 homodimerization and the binding of the STAT1 homodimers to GAS elements in gene promoter regions (5). Type III IFN signaling is similar to type I IFN signaling, involving activation of TYK2 and JAK1, recruitment of STAT1 and STAT2 and the formation of an ISGF3 complex which mediates gene transcription (20, 21) (Figure 1).

IFN responses are regulated by a group of 9 (in humans and mice) transcription factors, called IRFs. IRF proteins all contain an amino-terminal DNA binding domain that recognizes a consensus DNA sequence element called the interferon-stimulated response element (ISRE), present in the genes encoding IFNs and ISGs (5). IRFs have differing roles in regulation of IFN responses. For instance, IRF3 mediates

downstream signaling relayed by the adaptor proteins TRIF, MAVS, and STING to induce the production of type I IFNs (22). In contrast, IRF2 attenuates IRF3-mediated transcriptional activation (23). The effects of IRFs, however, are not necessarily limited to their roles in the immune response. The actions of several of the individual IRFs in the heart have been described, and these actions are summarized in the relevant section later in this review.

IFNs mediate their effects by stimulating the induction of several hundred ISGs (24), which can have unique and overlapping functions. Teleologically, the end effects of IFNs and IFN genes can be considered as ways in which an infected cell can limit the damage caused to itself and to neighbouring cells by an invading microbe. For instance the ISG, protein kinase R (PKR) is a stress induced kinase that restricts protein synthesis via phosphorylation of eukaryotic initiation factor 2 α (eIF2 α) (25). One of the most strongly induced ISGs is the ubiquitin-like (Ubl) protein ISG15 (ISG15; also called interferon-induced 15 kDa protein). ISG15 limits the cellular damage caused by viral myocarditis (26); whereas our group recently reported induction and a pathogenetic role of ISG15 in the adverse ventricular remodeling that occurs in response to pressure overload (27). These findings illustrate the divergent effects of IFN-related pathways in the presence or absence of microbial infection, and they are elaborated upon later. In addition to its role in modulating cellular and viral protein synthesis, the IFN response also regulates the host response to viral infection by stimulating the upregulation of major histocompatibility complex class I and II antigens (28, 29), promoting programmed cell death (30–32), regulating cellular differentiation (33), suppressing angiogenesis (34), and activating other immune cells (35, 36).

The type I IFN response and the heart

Induction of a type I IFN response has been reported to occur in both ischemic (37) and non-ischemic (27) cardiomyopathy, as well as in viral myocarditis (38). However, the contributions of the type I IFN response (being ostensibly protective or detrimental) depend on the underlying cause of the IFN response. In the context of viral infection, induction of type I IFNs has a largely protective effect (38), whereas in the absence of infection and in the setting of ischemia (37), or pressure overload (27), the type I IFN response may have deleterious consequences. Evidence of the direct cardiac effects of type I IFNs themselves can be sought through the study of genetic conditions and through clinical experience with therapeutic use of recombinant IFNs.

Cardiac involvement in monogenic and autoimmune interferonopathies

The monogenic type I interferonopathy Aicardi-Goutières syndrome (39) may present with an inflammatory cardiomyopathy (40); and type I IFNs have also been implicated in the pathogenesis of autoimmune congenital heart block (41). That being said, cardiac disease is not always a feature of

interferonopathy. Cardiac involvement is not, for instance, a common occurrence in STING-associated vasculopathy with onset in infancy (SAVI). Likewise, whereas systemic lupus erythematosus (SLE) is associated with both an IFN gene signature (42) and an increased risk of cardiovascular disease (CVD) (43), there are insufficient data to establish a causal association between the two (44).

Adverse cardiac effects of recombinant IFNs

Recombinant human IFN β 1b (e.g., Betaseron) is approved for the management of multiple sclerosis. The product monograph states that there is no evidence of a direct cardiotoxic potential of Betaseron, although cases of cardiomyopathy have been reported (45). Recombinant IFN α 2b (e.g., INTRON A) has regulatory approval for the management of chronic hepatitis C, chronic active hepatitis B, chronic myelogenous leukemia, multiple myeloma, non-Hodgkin's lymphoma, malignant myeloma, AIDS-related Kaposi's sarcoma, hairy cell leukemia, basal cell carcinoma, and condylomata acuminata (46). The product monograph for INTRON A states that adverse reactions associated with the cardiovascular system are mostly correlated with pre-existing CVD and prior cardiotoxic therapy, although transient reversible cardiomyopathy has been reported rarely in patients without prior evidence of cardiac disease (46). The interpretation from this experience of the use of recombinant IFNs for other indications is that the likelihood of deleterious cardiac effects of type I IFNs in otherwise normal hearts is relatively low. However, their effects in the presence of concurrent cardiac illness may be more notable. This is perhaps best exemplified by a report describing the role of IRF3 and type I IFNs in the response to myocardial infarction (MI) (37).

IRF3 and the type I IFN response to MI

In a landmark study published in 2017, King and co-workers used single cell RNA sequencing (scRNA-seq) to profile leukocytes isolated from the hearts of mice after MI (37). In doing so, they observed that a subtype of cardiac macrophages was characterized by IRF3/type I IFN activation, and that disruption of either IRF3 or IFNAR signaling resulted in improved survival following MI, decreased inflammation and improved cardiac function (37). The authors concluded that the high level of cell death that occurs in MI results in disruption of the compartmentalization of DNA in the cell nucleus and mitochondria and interferes with the housekeeping actions of self-DNases. This leads to the release of damage signals, especially dsDNA from dying cells (37). dsDNA, in turn, is sensed by cGAS in infiltrating phagocytes leading to activation of an IRF3-dependent type I IFN response (37). Secreted IFNs then diffuse in the local microenvironment, binding to IFNARs on neighbouring cells and amplifying the type I IFN response through induction of ISGs (37). In our opinion, this postulated mechanism most clearly exemplifies how type I IFNs may be induced during myocardial injury and how the type I IFN response may, in turn, contribute to adverse cardiac outcomes.

ISG15 in viral myocarditis and pressure overload

Whereas the example above illustrates the potentially deleterious effects of the type I IFN response in MI, type I IFNs are protective against viral myocarditis. For instance, mice deficient in IFNAR are susceptible to coxsackievirus B3 (CVB3) infection (47), and mice deficient in IFN- β experience exacerbated CVB3-induced myocarditis (48). The example of ISG15 illustrates how a downstream effector ISG of the type I IFN response also may play a context-dependent role in the protection from or development of cardiac injury. ISG15 is a Ubl protein and, in this role, once it is induced ISG15 can post-translationally modify lysine residues on actively translated proteins, including viral proteins (49) (Figure 3). The post-translational modification of proteins by conjugation with ISG15 is termed, ISGylation. In addition to its intracellular effects, ISG15 also exists in an intracellular free form and it can also be secreted. In its secreted form, ISG15 binds to its receptor, lymphocyte function-associated antigen 1 (LFA-1) expressed especially by T cells and NK cells, where it stimulates IFN- γ production (50, 51) (Figure 3). Absence of ISG15 has been reported to exacerbate CVB3 myocarditis (26), and this effect was also mimicked in mice lacking ubiquitin-activating enzyme E1-like (UBE1L), the E1-activating enzyme necessary for protein ISGylation (26). The authors of that article attributed the protective actions of ISG15 to the ISGylation of CVB3 2A protease, limiting CVB3-induced cleavage of host eukaryotic initiation factor 4 γ (eIF4G) in cardiomyocytes, which ordinarily promotes viral infection by restricting host cell protein translation (26). A recent study, further illuminated the role of ISG15 in viral myocarditis, concluding that induction of ISG15 in myocarditis functions to counter cardiac atrophy and dysfunction by increasing the heart's metabolic capacity through downregulation of cardiac glycolysis and enhancing the respiratory activity of mitochondria (52).

Viral infection, however, is not the only cause of cardiomyocyte ISG15 induction. For instance, Maier and coworkers reported that cardiomyocytes with constitutively active I κ B kinase/NF κ B signaling exhibited a type I IFN response that is characterized by activation of the ISG15 pathway (53). In the absence of viral infection, the conjugation of ISG15 to actively translated host proteins can affect several cellular processes including cytoskeletal dynamics, DNA damage responses, cytokine release, and immune modulation (54). In recent work by our group, we set out to determine the mechanism(s) by which proinflammatory CCR2-expressing macrophages contribute to pressure overload-induced ventricular remodeling (27). We found that exposure of cardiomyocytes to the secreted products of CCR2+ cardiac macrophages isolated from mouse hearts, induced a profound type I IFN response, characterized by ISG15 induction. Cardiac ISG15 induction was also observed in mouse hearts after transverse aortic constriction (TAC) or following infusion with angiotensin II, the left ventricles (LVs) of uninephrectomized rats treated with deoxycorticosterone acetate (DOCA) salt, and the right ventricles of rats after pulmonary artery banding (27). We observed that, in pressure overload, ISG15 induction results in the ISGylation of newly translated cardiomyocyte proteins, including the myofibrillar protein, filamin-C (27), and that absence of ISG15 attenuated adverse ventricular remodeling after TAC (27). Interestingly, in that

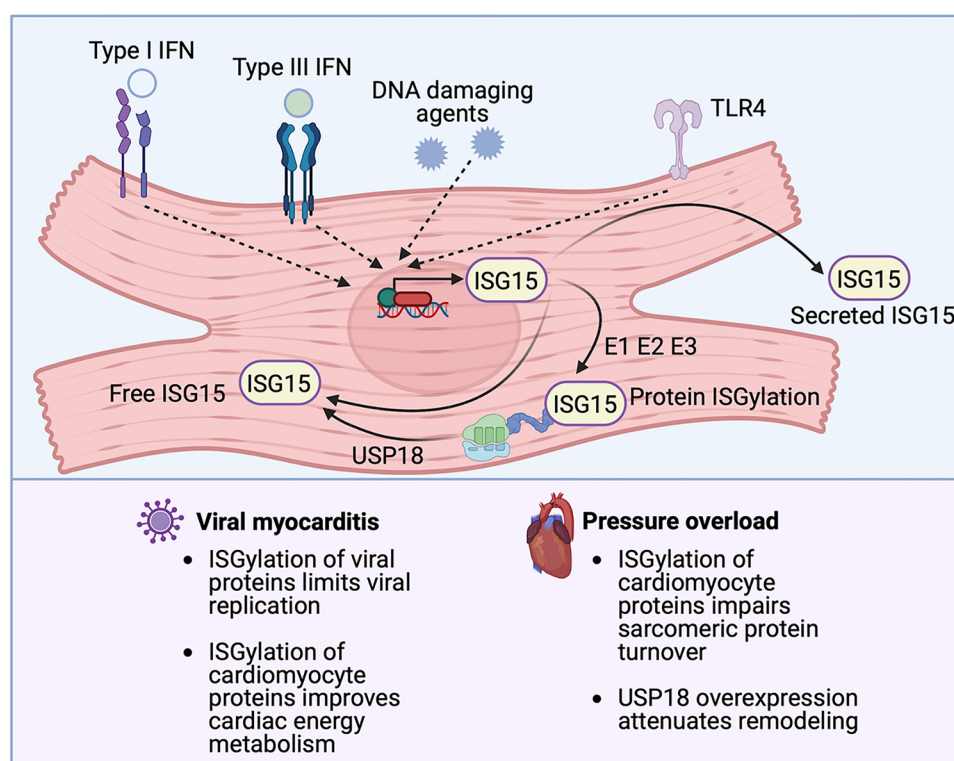


FIGURE 3

The emerging role of ISG15 in heart disease. ISG15 is one of the most strongly inducible interferon (IFN) stimulated genes, the expression of which can be triggered by IFNs themselves, DNA damaging agents or TLR signaling. ISG15 exists in 3 forms, an intracellular free form, a secreted form and a protein-bound form. ISG15 binds to newly synthesized viral or host proteins through an energy consuming process, termed ISGylation, that requires E1-activating, E2-conjugating, and E3-ligating enzymes. Removal of ISG15 from proteins (de-ISGylation) is mediated by the protease USP18. ISG15 induction protects against viral myocarditis, likely through the ISGylation of both viral and cardiomyocyte proteins. Conversely, in the absence of microbial infection, ISG15 induction can have detrimental effects, as has been observed in pressure overload, where the ISGylation of newly synthesized cardiomyocyte proteins impairs sarcomeric protein turnover.

study, we also found cardiac ISG15 levels to be markedly reduced in *Ifnar1* deficient mice even in the absence of pressure overload (27). This observation illustrates that constitutively expressed IFNs can have important physiological roles even in the absence of induction and even though they are present at very low levels (55).

Protein ISGylation is mediated by an energy-consuming process involving E1-activating enzymes, E2-conjugating enzymes, and E3-ligating enzymes and it is reversed by an ISG15-specific protease called ubiquitin-specific protease 18 (USP18), which itself is an ISG (56–58) (Figure 3). Whereas we reported a pathogenetic role for protein ISGylation in pathological ventricular hypertrophy, Ying and coworkers described a protective effect of USP18 overexpression, which would be expected to reverse protein ISGylation (59). In brief, the authors observed that cardiomyocyte-specific overexpression of USP18 attenuated myocardial hypertrophy, fibrosis, ventricular dilatation, and ejection fraction decline induced by aortic banding, whereas USP18 knockout exacerbated remodeling (59). The authors attributed the cardioprotective effects of USP18 to inhibition of transforming growth factor β -activated kinase 1 (TAK1)/MAPK/JNK signaling (59). They had focused on this pathway because USP18 had previously been reported to deubiquitinate TAK1 (60), whereas the polyubiquitination of TAK1 is important for its autoactivation and

downstream activation of p38 MAPK/JNK signaling (61). Other studies, though, have reported that USP18 is specific for ISG15 and that USP18 does not remove ubiquitin from substrate proteins (62, 63). Accordingly, it is feasible that the phenotypes observed in the USP18 overexpressing/knockout mice were mediated through altered protein ISGylation, although this possibility was not explored in the original report (59).

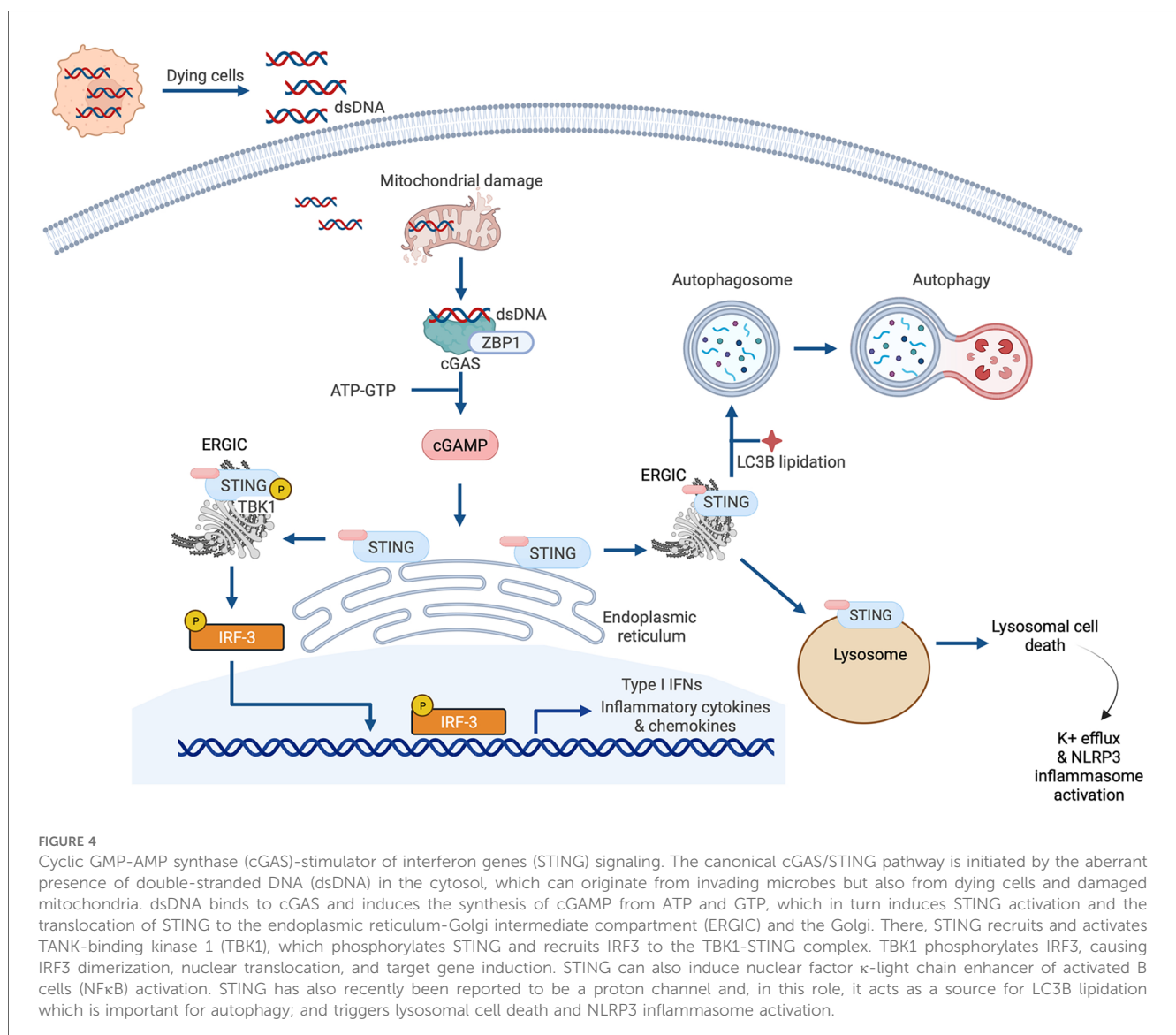
Lastly, whereas the role of ISG15 in the defence against viral infection has been known about for decades (64), its biological function in this capacity has gained increasing attention of late because of the involvement of ISG15 in COVID-19, with potentially intracellular and extracellular proviral and antiviral effects (65). The contribution of an insufficient or augmented IFN response to COVID-19 severity and its potential cardiac complications is discussed later in this review.

The emerging role of cGAS-STING pathway activation in the pathogenesis of heart disease

The earlier summarized study by King et al. that reported the importance of IRF3 and the type I IFN response in MI described

a central role for cGAS-STING signaling in myocardial injury (37). This is one of several recent studies espousing the significance of cGAS-STING pathway activation in cardiac disease that have emerged since the original description of the pathway in 2013 (66, 67). Briefly, the cGAS-STING signaling pathway exists to mediate the immune response to displaced dsDNA which can originate from invading microorganisms, dead cells, extracellular vesicles, or leakage of DNA from damaged mitochondria (Figure 4). dsDNA binds to cGAS and activates the synthesis of the second messenger 2'3' cyclic GMP-AMP (cGAMP) from ATP and GTP. cGAMP, in turn, binds to the active pocket site of the dimeric adaptor protein STING, causing STING activation and downstream signaling (68). Once it is activated, STING translocates from its residing place in the endoplasmic reticulum (ER) to the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) and the Golgi where it recruits and activates TANK-binding kinase 1 (TBK1), which in turn phosphorylates STING [on serine residue 366 (Ser366) in humans, Ser365 in mice], and recruits IRF3 to the TBK1-STING

complex (22, 69) (Figure 4). TBK1 phosphorylates IRF3, causing IRF3 dimerization, nuclear translocation and induction of its target genes (69), including type I IFNs, ISGs and inflammatory cytokines (70) (Figure 4). In addition to this canonical mechanism of gene induction by cGAS-STING, STING can also induce NFκB activation (68). The role of TBK1 in NFκB activation is unclear. It has been suggested that TBK1 is dispensable for STING activation of NFκB, and that this process requires TAK1 and IκB kinase (IKK) complexes in myeloid cells (71). However, other authors have reported that TBK1 recruitment is necessary for STING-mediated NFκB activation (72). Aside from (and independent of) its role in mediating the induction of IFNs and cytokines, STING also plays an important role in autophagy induction (73), and inflammasome activation (74). For instance, when STING binds cGAMP and translocates to the ERGIC, the STING-containing ERGIC acts as a source for non-canonical LC3B lipidation which is important for the biogenesis of autophagosomes (73) (Figure 4). STING-induced autophagosome formation is dependent on a direct interaction



between STING and WD repeat domain, phosphoinositide interacting 2 (WIPI2) (75). Separately, activated STING traffics to the lysosome where it triggers membrane permeabilization and lysosomal cell death, and potassium efflux which promotes NLRP3 inflammasome activation (74) (Figure 4). Interestingly, human STING has also recently been reported to act as a proton channel, and its effects in both L3B lipidation and inflammasome activation have been attributed to this property (76).

Experimental studies implicating cGAS-STING pathway activation in the pathogenesis of heart disease are summarized in Table 1. Some of the findings from these studies are elaborated upon below.

cGAS-STING in MI

In 2018 Cao and coworkers reported that MI caused by ligation of the left anterior descending (LAD) artery induced upregulation of the cGAS-STING pathway, which sustains the inflammatory “M1-like” macrophage phenotype (77). Furthermore, inactivation of the pathway through knockout of cGAS prompted a more “M2-like” macrophage phenotype that was accompanied by improved wound healing, enhanced angiogenesis, diminished remodeling, and improved survival (77). Similarly, treatment of mice with the STING antagonist H-151 has been reported to improve outcomes after experimental MI (82, 83).

cGAS-STING in non-ischemic cardiomyopathy

Zhang and coworkers reported STING upregulation in heart tissue of humans with dilated cardiomyopathy or HCM, and in

the hearts of mice with pressure overload induced by aortic banding (90). In that study, knockout of STING attenuated pathological hypertrophy and ejection fraction decline induced by aortic banding (90). Similarly, Hu et al. also observed activation of the cGAS-STING pathway in mice with ventricular remodeling caused by TAC, with a preservation of LV function (and improved survival) when cGAS was knocked down using adeno-associated virus 9 (AAV9) gene transfer of short hairpin RNA (shRNA) (78).

cGAS-STING in diabetes and in sepsis

Yan et al. reported that oxidative damage-induced mtDNA leak was accompanied by cGAS-STING pathway activation in the hearts of diabetic high fat diet-fed mice, and that AAV9-mediated knockdown of STING with shRNA preserved cardiac function (84). In a model of sepsis-induced cardiac injury, Li et al. observed that global STING knockout attenuated LV systolic dysfunction and improved survival in mice injected with LPS (91).

Noncanonical actions of cGAS-STING in the heart

The above examples, and those additional studies summarized in Table 1, illustrate how a substantial body of literature has arisen in recent years indicating that cGAS-STING pathway activation takes place in both ischemic and non-ischemic cardiomyopathy and that blockade of cGAS-STING signaling improves cardiac outcomes in experimental models. More recently still, new fundamental insights into cGAS-STING biology have emerged through the study of the actions of the pathway in the heart. For

TABLE 1 Experimental studies implicating the cGAS-STING pathway in heart disease.

Years	Disease or experimental context	Summary of principal findings	Citation
2017	Myocardial infarction	Mice with functionally deficient STING mimic IRF3 knockout mice in their gene expression patterns after MI	(37)
2018	Myocardial infarction	cGAS knockout improved survival in mice after MI, decreased pathological remodeling, enhanced angiogenesis, and preserved contractile function	(77)
2020	Pressure overload	AAV9-mediated knockdown of cGAS with shRNA improved survival, preserved LV contractility, and attenuated pathological remodeling in mice after TAC	(78)
2020	High fat diet	High fat diet upregulated cGAS-STING and augmented cardiac remodeling in Akt2-AMPK double knockout mice, and cGAS-STING inhibition attenuated cardiomyocyte contractile dysfunction	(79)
2020	Alzheimer's disease	Knockdown of cGAS or STING negated the beneficial effects of melatonin on neonatal cardiomyocyte mitophagy and apoptosis in response to APP/PS1 mutation	(80)
2020	Cigarette smoke	cGAS-STING inhibition rescued smoke-induced contractile dysfunction in wildtype and <i>Becn1</i> haploinsufficient (<i>Becn1</i> ^{+/-}) neonatal cardiomyocytes, except cGAS inhibition in <i>Becn1</i> ^{+/-} cardiomyocytes	(81)
2022	Reperfused myocardial infarction	Small molecule STING inhibition decreased infarct expansion, attenuated cardiac function decline, and reduced myocardial hypertrophy	(82)
2022	Myocardial infarction	Small molecule STING inhibition preserved cardiac function and attenuated fibrosis in mice with MI	(83)
2022	Diabetic cardiomyopathy	AAV9-mediated knockdown of STING with shRNA preserved cardiac function and hypertrophy and alleviated cardiac pyroptosis in streptozotocin-diabetic high fat diet-fed mice	(84)
2023	Doxorubicin-induced cardiotoxicity	AAV9-mediated knockdown of STING with shRNA improved survival and cardiac function and reduced inflammation in mice with doxorubicin cardiotoxicity	(85)
2023	Cardiac transplantation	Graft survival was prolonged in donor hearts from cGAS knockout mice	(86)
2023	Doxorubicin-induced cardiotoxicity	Global deficiency of cGAS, STING or IRF3 each ameliorated doxorubicin-induced cardiotoxicity in mice; and endothelial-specific STING deficiency attenuated cardiotoxicity and endothelial dysfunction	(87)
2023	Doxorubicin-induced cardiotoxicity	ZBP1 stabilizes Z-form mtDNA and cooperates with cGAS to induce type I IFN signaling and cardiotoxicity induced by doxorubicin	(88)
2023	Cholesterol metabolism	Carnitine acetyltransferase depletion induced mtDNA stress and a cardiomyocyte innate immune response mediated by cGAS-STING	(89)

cGAS, cyclic GMP-AMP synthase; STING, stimulator of interferon genes; MI, myocardial infarction; IRF, interferon regulatory factor; AAV9, adeno-associated virus 9; shRNA, short hairpin RNA; LV, left ventricle; TAC, transverse aortic constriction; AMPK, AMP-activated protein kinase; APP, amyloid precursor protein; PS1, presenilin 1; ZBP1, Z-DNA binding protein 1; mtDNA, mitochondrial DNA.

example, one recent study reported that cGAS interacts with the innate immune sensor protein Z-DNA binding protein 1 (ZBP1) to promote type I IFN responses and cardiotoxicity (88). Briefly, because mtDNA is circular and lacks free ends it cannot rotate to relieve torsional stress. As a result, mitochondrial genome instability promotes the accumulation of a form of DNA called Z-DNA which differs from classical Watson-Crick B-DNA in its conformation including (but not limited to) that Z-DNA is a left-handed double helix, and B-DNA is a right-handed double helix. Mitochondrial Z-DNA is stabilized by ZBP1 which nucleates a complex that contains cGAS, as well as the mediators of cell death and inflammation, receptor-interacting protein 1 (RIPK1) and RIPK3 (88). This, in turn, augments STAT1 phosphorylation, and induces type I IFN signaling that is dependent on ZBP1, STING and IFNAR (88). Illustrating the importance of the interaction between ZBP1 and cGAS in mtDNA-sensing and downstream signaling, mice lacking ZBP1, STING or IFNAR1 were protected from doxorubicin-induced cardiotoxicity (88). Interestingly though, ZBP1 is not required for sensing of cytosolic B-form mitochondrial DNA (88). Separately, in another recent study, Mao et al. implicated cGAS-STING activation as an important mediator of the mechanisms by which

altered cholesterol metabolism may affect innate immune responses in the heart (89). In that study, the investigators reported that depletion of carnitine acetyltransferase (CRAT) from cardiomyocytes promoted cholesterol catabolism, and accumulation of bile acid and the intermediate 7 α -hydroxyl-3-oxo-4-cholestenoic acid (89). This, in turn, induced mitochondrial stress and cGAS-STING-dependent type I IFN responses, which contributed to myocardial inflammation and heart failure (89).

IFN- γ

IFN- γ in the heart arises from infiltrating inflammatory cells, primarily CD4+ and CD8+ T cells and NK cells (92, 93), with some contribution from macrophages (92, 94) (Figure 5). Interaction between T cells and macrophages is important in driving IFN- γ production by each cell-type, and IFN- γ plays a central role in mediating crosstalk between innate and adaptive immune cell populations (92, 95). Cardiac IFN- γ levels are increased in a range of different diseases including myocarditis (96), Chagas disease cardiomyopathy (97–99), MI (100),

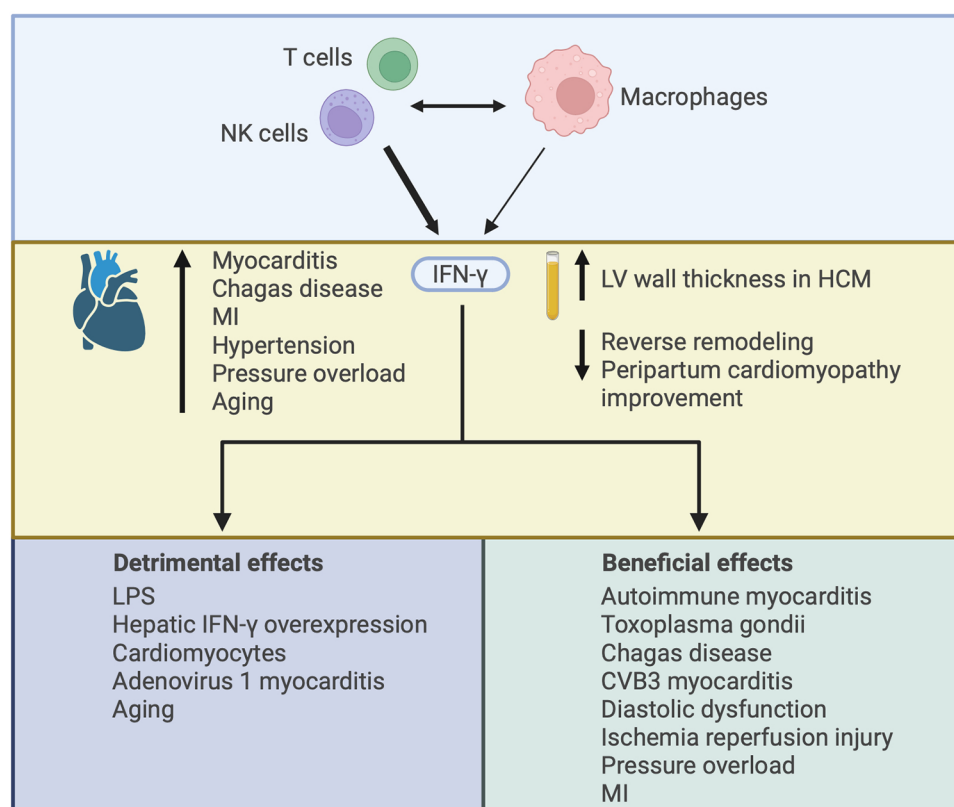


FIGURE 5

Illustrating the varied effects of interferon- γ (IFN- γ) in the heart. IFN- γ is mainly produced by CD4+ and CD8+ T cells and NK cells, with some contribution from macrophages. Interaction between adaptive and innate immune cells can drive IFN- γ production by each cell-type. IFN- γ expression in the heart has been described in several disease settings and increased plasma levels of IFN- γ have been associated with worsened cardiac outcomes. The increase in IFN- γ may contribute to cardiac injury or it may be compensatory, with both detrimental and beneficial effects of IFN- γ described according to the nature of IFN- γ augmentation or deficiency and the context in which it has been studied. HCM, hypertrophic cardiomyopathy; MI, myocardial infarction; LPS, lipopolysaccharide.

hypertensive heart disease (101), pressure overload (94), and aging (102) (Figure 5). In comparison to the type I IFNs, a much larger body of literature exists attesting to the actions of IFN- γ in the heart, with overall conflicting reports describing both beneficial and detrimental context-dependent effects of IFN- γ . These studies are summarized in Table 2 and Figure 5, and some of the key observations are discussed below.

Studies reporting deleterious actions of IFN- γ in the heart

Transgenic mice that overexpress IFN- γ in their livers, and thus with high circulating serum levels of IFN- γ , have been reported to develop a chronic active myocarditis and cardiomyopathy characterized by accumulation of CD4+ and CD8+ T cells, macrophages and dendritic cells (105). This was accompanied by upregulation in the expression of proinflammatory cytokines including TNF α , IL-12, CCL2 and CCL3 (105), illustrating the role of IFN- γ in inflammatory gene induction. Furthermore, in patients with LV assist device (LVAD) implantation, low serum IFN- γ (and TNF α) predicted cardiac improvement (116). This is interesting because, in experimental studies, co-exposure of cardiomyocytes to IFN- γ and TNF α has also been reported to induce mitochondrial dysfunction and nitro-oxidative stress (99).

These findings suggesting deleterious actions of IFN- γ in the heart are supported by several other studies in experimental animals (92), isolated atria (117), cultured cardiomyocytes (99), and cultured cardiac fibroblasts (118), reviewed in (95). Given the production of IFN- γ by infiltrating adaptive immune cells, and the role of IFN- γ in orchestrating the interaction between innate and adaptive immune cells, this seems teleologically appropriate. However, several other studies have described a protective role for IFN- γ in the heart.

Studies reporting protective actions of IFN- γ in the heart

IFN- γ knockout mice have been reported to develop worsened hypertrophy, cardiac fibrosis and cardiac dysfunction after TAC (94). This has been attributed to an essential role of IFN- γ in Stat5-dependent activation of phosphoinositide 3-kinase/Akt signaling during compensatory hypertrophy (94). Cardiac inflammation has also been reported to be increased in IFN- γ knockout mice injected with cardiac myosin and accompanied by a constrictive pericarditis (111). Furthermore, cardiac hypertrophy and diastolic dysfunction were exacerbated in IFN- γ knockout mice with aldosterone, uninephrectomy and salt water feeding (114), whereas recombinant IFN- γ attenuated cardiac

TABLE 2 Experimental studies reporting the detrimental or beneficial effects of IFN- γ in the heart.

Years	Disease or experimental context	Reported actions of IFN- γ	Citation
Detrimental effects of IFN-γ			
1998	Rat papillary muscle	Cardiodepressant effect of IFN- γ in the presence of LPS	(103)
2001	Isolated retrograde-perfused rat hearts	Augmented depression of inotropic and lusitropic effects of LPS and IFN- γ in aged hearts	(104)
2007	Hepatic IFN- γ overexpression	Chronic active myocarditis and cardiomyopathy in IFN- γ overexpressing mice	(105)
2012	Hepatic IFN- γ overexpression	TNF α knockout attenuated myocarditis and cardiomyopathy in IFN- γ overexpressing mice	(106)
2012	Neonatal rat ventricular myocytes	Cardiac myocyte atrophy induced by recombinant IFN- γ due to degradation of myosin heavy chain protein	(107)
2015	Mouse adenovirus 1 (MAV-1)	Attenuated myocarditis with neutralizing antibody mediated IFN- γ depletion	(96)
2021	AC16 cardiomyocytes	Co-incubation with IFN- γ and TNF α induced cardiomyocyte mitochondrial dysfunction and nitro-oxidative stress	(99)
2023	Aging	IFN- γ response signature in aging mouse hearts mimicked by recombinant IFN- γ treatment of induced pluripotent stem cell derived cardiomyocytes, accompanied by reductions in oxidative phosphorylation and glycolysis	(102)
Beneficial effects of IFN-γ			
2001	Autoimmune myocarditis	Exacerbated cardiac α -myosin heavy chain induced autoimmune myocarditis in IFN- γ receptor knockout mice	(108)
2001	Toxoplasma gondii	Augmented accumulation of Toxoplasma gondii in the hearts of IFN- γ knockout mice	(109)
2001	Chagas disease	Augmented cardiac Trypanosoma cruzi parasitism in IFN- γ knockout mice	(110)
2004	Autoimmune myocarditis	Constrictive pericarditis and augmented myocarditis in IFN- γ knockout mice injected with cardiac myosin	(111)
2004	Viral myocarditis	Worsened CVB3-induced myocarditis in IFN- γ knockout mice	(112)
2005	PGF(2 α)-treated rat cardiac myocytes and abdominal aortic constriction	Recombinant IFN- γ attenuated myocardial hypertrophy	(113)
2012	Aldosterone infusion, uninephrectomy and 1% saline water	Augmented LV hypertrophy and worsened diastolic dysfunction in IFN- γ knockout mice	(114)
2013	Porcine cardiopulmonary bypass-associated myocardial ischemia-reperfusion injury	Preconditioning with IFN- γ improved recovery of ventricular function after ischemia reperfusion injury	(115)
2018	Pressure overload	Worsened hypertrophy, cardiac fibrosis and dysfunction in IFN- γ knockout mice after TAC	(94)
2019	Left anterior descending artery ligation	IFN- γ orchestrated the sequential cellular immune response after MI and IFN- γ knockout impaired cardiac function and survival after MI	(100)

Table does not include studies reporting solely an association of IFN- γ with cardiac or atherosclerotic disease, or the role of IFN- γ in the immune response to transplantation.

IFN- γ , interferon- γ ; LPS, lipopolysaccharide; TNF α , tumor necrosis factor α ; MAV-1, mouse adenovirus 1; CVB3, coxsackievirus B3; PGF(2 α), prostaglandin F2 α ; LV, left ventricle, TAC, transverse aortic constriction; MI, myocardial infarction.

hypertrophy in rats with abdominal aorta banding (113). Other reports also describing beneficial actions of IFN- γ in the heart are also summarized in Table 2.

In sum, cardiac IFN- γ is increased in several different disease states. Its role in these diseases may contribute to their pathogenesis, or it may be compensatory, with experimental studies reporting both deleterious and protective actions of IFN- γ . These actions appear to be dependent on the models studied, timing of intervention, mechanism of upregulation or inhibition, and the experimental endpoints employed.

Interferon regulatory factors

The IRFs are a family of 9 transcription factors that play an important role in the immune response, also regulating other cellular processes. IRF1 regulates gene expression by binding to ISREs in their promoter regions (119) and, although it is best studied as a transcriptional activator (119), IRF1 can also function as a transcriptional repressor (120). IRF2 competitively inhibits IRF1-mediated gene transcription (121). IRF3, IRF5 and IRF7 are important for the production of type I IFNs in response to PRR-mediated signaling (122). IRF9 regulates IFN-induced gene expression, and IRF4, IRF5 and IRF8 control myeloid cell development and responses (122). IRF6 is important for early development (123). Most of the IRFs have been studied individually for their role in the heart, and these actions are summarized below and in Table 3.

Several of these studies were published by the same team in 2013–2014, and followed a similar pattern of investigation involving: exploration of change in IRF protein levels in diseased

hearts; cardiac-specific IRF overexpression and global IRF knockout; and elucidation of a cellular mechanism underlying the beneficial or detrimental cardiac effects of IRF overexpression and knockout (124, 128, 129, 132, 134, 135).

IRF1

IRF1 expression has been reported to be reduced in the hearts of humans with dilated cardiomyopathy or hypertrophic cardiomyopathy, whereas in mice with pressure overload caused by banding of the thoracic aorta there was an early upregulation of IRF1 after 3–7 days, followed by a reduction in IRF1 protein in the heart by weeks 4–6 (124). Cardiac-specific IRF1 overexpression exacerbated pressure overload-induced hypertrophy, ventricular dilatation and dysfunction, whereas cardiac hypertrophy was attenuated in IRF1 knockout mice and rats (124). These prohypertrophic effects of IRF1 were attributed to induction of inducible nitric oxide synthase (iNOS) caused by binding of IRF1 to the promoter region of the iNOS gene (124). IRF1 has also been implicated in the pathogenesis of cardiac dysfunction that can occur because of chronic kidney disease (CKD), termed cardiorenal syndrome type 4 (125). In that study, the authors reported that high phosphate levels in CKD impair myocardial energy metabolism by downregulating the transcription of the master regulator of mitochondrial biogenesis, peroxisome proliferator-activated receptor gamma activator 1-alpha (PGC1 α) (125). Mechanistically, high phosphate was observed to epigenetically regulate the expression of IRF1 by inducing acetylation of histone protein H3 on lysine residue 9 (H3K9) (125). IRF1 has a repressor domain and, in some circumstances, can bind to the IRF response element of target genes to repress gene expression (136, 137). In the case of

TABLE 3 Experimental studies exploring the actions of interferon regulatory factors (IRFs) in cardiac disease.

IRF	Years	Disease or experimental context	Reported actions of IRFs	Citation
IRF1	2014	Pressure overload	Cardiac IRF1 overexpression exacerbated hypertrophy, ventricular dilatation and dysfunction, whereas IRF1 knockout attenuated cardiac hypertrophy	(124)
IRF1	2020	Cardiorenal syndrome type 4	IRF1 mediated cardiac PGC1 α downregulation and consequent dysfunction of myocardial energy metabolism	(125)
IRF2	2021	Myocardial infarction	IRF2 contributed to cardiac dysfunction in MI by inducing gasdermin D-mediated pyroptosis	(126)
IRF3	2011	Angiotensin II induced cardiac fibrosis	IRF3 knockout attenuated cardiac fibrosis	(127)
IRF3	2013	Aortic banding	IRF3 knockout exacerbated cardiac hypertrophy, whereas cardiac IRF3 overexpression attenuated it	(128)
IRF3	2017	Myocardial infarction	MI induced activation of an IRF3-type I IFN axis in cardiac macrophages which was caused by release of DNA from damaged cells, and which impaired cardiac function	(37)
IRF4	2013	Aortic banding	Cardiac specific overexpression of IRF4 exacerbated hypertrophy, fibrosis, and dysfunction and IRF4 knockout attenuated cardiac hypertrophy	(129)
IRF5	2014	Myocardial infarction	Nanoparticle-delivered siRNA against IRF5 supported infarct healing and attenuated heart failure after MI	(130)
IRF5	2019	Viral myocarditis	IRF5 interference attenuated viral myocarditis	(131)
IRF7	2014	Aortic banding	Cardiac specific overexpression of IRF7 attenuated hypertrophy, fibrosis, and dysfunction and IRF7 knockout augmented cardiac hypertrophy and fibrosis	(132)
IRF7	2022	Cardiac autoinflammation	IRF7 knockout attenuated cardiac autoinflammation induced by ADAR1 inactivation	(133)
IRF8	2014	Aortic banding	Cardiac specific overexpression of IRF8 attenuated hypertrophy and fibrosis, and IRF8 knockout augmented cardiac hypertrophy and fibrosis	(134)
IRF9	2013	Aortic banding	Cardiac-specific overexpression of IRF9 attenuated cardiac hypertrophy whereas hypertrophy, fibrosis and cardiac dysfunction were augmented in IRF9 knockout mice	(135)

Table does not include studies reporting solely an association of IRFs with cardiac or atherosclerotic disease, or the role of IRFs in the immune response to transplantation. No experimental studies were identified that examined the role of IRF6 in cardiac disease.

IRF, interferon regulatory factor; PGC1 α , peroxisome proliferator-activated receptor gamma activator 1-alpha; MI, myocardial infarction; siRNA, short interfering RNA; ADAR1, adenosine deaminase acting on RNA-1.

cardiorenal syndrome type 4, IRF1, induced by high phosphate, was observed to bind directly to the promoter region of the PGC1 α encoding gene inhibiting PGC1 α transcription (125).

IRF2

IRF2 levels have been reported to be increased after experimental MI, whereas lentiviral mediated silencing of IRF2 with shRNA attenuated cardiac dysfunction after MI, an effect attributed to the role of IRF2 in gasdermin D-mediated pyroptosis (126).

IRF3

Several studies have explored the effects of IRF3 in the heart. In 2011, Tsushima and coworkers reported that IRF3 knockout attenuated cardiac fibrosis and ventricular chamber shrinkage in mice infused with angiotensin II, whereas cardiac hypertrophy was unaffected (127). In that study, the authors attributed IRF3 activation by angiotensin II in cardiac fibroblasts to be mediated by ERK signaling rather than canonical TBK1/IKK signaling (127). Lu and coworkers performed aortic banding in IRF3 knockout mice and in mice with cardiac-specific IRF3 overexpression, observing that IRF3 knockout exacerbated cardiac hypertrophy and IRF3 overexpression attenuated it, and concluding that IRF3 is a negative regulator of pathological cardiac hypertrophy (128). They attributed this effect to an interaction between IRF3 and ERK2, which inhibited ERK1/2 signaling (128). The authors also observed that IRF3 levels are increased early after aortic banding and return to basal levels by day 28, whereas IRF3 is also upregulated in human failing hearts (128). Considering the more recent findings of King et al, using scRNA-seq (37), it seems likely that the early increases in cardiac IRF3 levels in mice after aortic banding and in human heart failure may represent accumulation of cardiac macrophages, which is known to occur early after aortic banding (138).

IRF4

Jiang et al. reported that IRF4 is downregulated in human dilated cardiomyopathy, in mouse hearts 4 and 8 weeks after aortic banding, and in cardiomyocytes exposed to angiotensin II or phenylephrine (129). They found that cardiac specific overexpression of IRF4 exacerbated pressure overload-induced hypertrophy, fibrosis, and dysfunction, whereas IRF4 knockout attenuated cardiac hypertrophy (129). The authors attributed this effect to a role for IRF4 in inducing the transcription of cAMP response element-binding protein (CREB) in cardiomyocytes, which is known to promote cardiac hypertrophy (139, 140).

IRF5

IRF5 plays a key role in macrophage polarization favoring an “M1-like” phenotype (141, 142). In 2014, Courties et al. used nanoparticle-delivered siRNA to silence IRF5 in infarct macrophages and observed that IRF5 knockdown attenuated the development of heart failure after MI in ApoE knockout mice (130). Whereas most IFNs and IFN-related pathways have shown to have protective roles in viral myocarditis, IRF5 may play a role in the pathogenesis of cardiac injury in this setting.

Specifically, Nie et al. observed upregulation of the TLR9-IRF5 pathway in the hearts of humans and mice with CVB3 myocarditis, and they observed that an AAAG-rich oligodeoxynucleotide that interferes with IRF5 alleviated myocarditis in CVB3-infected mice (131).

IRF7

Jiang et al. also studied the actions of IRF7, observing that IRF7 negatively regulates cardiac hypertrophy (132). Briefly, the authors reported that either angiotensin II or phenylephrine decreased IRF7 protein levels in neonatal rat cardiomyocytes, and IRF7 protein levels were also observed to be reduced in the hearts of mice 2 and 4 weeks after aortic banding (132). Cardiac specific IRF7 overexpression attenuated pressure overload-induced hypertrophy, fibrosis, and dysfunction, whereas IRF7 knockout augmented cardiac hypertrophy and fibrosis (132). These effects were attributed by the authors to binding of IRF7 to inhibitor of κ B kinase- β (IKK β) and consequent inactivation of NF κ B (132). Elsewhere, IRF7 expression levels have been reported to be markedly increased in the hearts of mice with CVB3 myocarditis (143). More recently, inactivation of adenosine deaminase acting on RNA-1 (ADAR1) (which acts as an RNA sensing inhibitor) was found to induce a late-onset autoinflammatory myocarditis, dilated cardiomyopathy, and heart failure (133). This phenotype was attenuated by IRF7 knockout, indicating that IRF7 is the principal mediator of cardiac autoinflammation induced by ADAR1 absence (133).

IRF8

When studying the cardiac effects of IRF8, Jiang and coworkers observed a reduction in cardiac IRF8 protein levels in the hearts of humans with dilated cardiomyopathy or hypertrophic cardiomyopathy, mice with pressure overload caused by aortic banding, and in neonatal rat cardiomyocytes incubated with angiotensin II or phenylephrine (134). Mice with cardiac-specific overexpression of IRF8 were resistant to hypertrophy and fibrosis induced by pressure overload, whereas either global- or cardiomyocyte-specific IRF8 knockout aggravated adverse remodeling (134). The investigators attributed this effect to an interaction between IRF8 and nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1) which inhibits the nuclear translocation of NFATc1 (134). The authors speculated that the interaction between IRF8 and NFATc1 may prevent dephosphorylation of NFATc1 by calcineurin which ordinarily promotes nuclear translocation and facilitates pathological hypertrophy (134, 144).

IRF9

Lastly, Jiang et al. also studied the effects of IRF9 in the heart (135). In that study IRF9 protein levels were observed to be increased in the hearts of mice 2 and 4 weeks after aortic banding and in neonatal rat cardiomyocytes exposed to angiotensin II or isoproterenol (135). Cardiac-specific overexpression of IRF9 attenuated cardiac hypertrophy whereas hypertrophy, fibrosis and cardiac dysfunction were augmented in IRF9 knockout mice (135). These effects were attributed by the authors to an action of IRF9 in competing with p300 for binding to the transcription activation

domain of myocardin (135), a transcriptional coactivator and inducer of cardiac hypertrophy (145).

The IFN response and the heart in COVID-19

The COVID-19 pandemic has shone the spotlight on the role that IFNs play in the defence against viral infection, and potentially also the contribution of IFNs to adverse outcomes. COVID-19 can result in several different cardiovascular complications including heart failure, arrhythmia, acute coronary syndrome, MI, myocarditis, and acute myocardial injury (146). Indeed, myocardial injury may be the most common extrapulmonary complication of COVID-19, affecting over 70% of those with severe disease (146). Most studies and commentaries discussing the role of IFNs in COVID-19 do not distinguish the cardiac effects specifically from systemic effects associated with severe disease or critical illness. Furthermore, whereas cardiac complications are common in severe COVID-19, the relative contributions of direct viral infection and the immune response to viral infection (including the IFN response) have not been disentangled. It is similarly challenging to disentangle the relative contributions of either individual IFNs or the IFN response from hyperinflammation in general (147, 148). Nevertheless, both a delayed persistent type I IFN response and diminished capacity to produce type I IFNs have been linked to COVID-19 severity (147). Thus, in COVID-19, the optimal IFN response is one that is finely balanced and dependent on host factors, stage and severity of disease and site of infection, amongst other factors (147, 148). For instance, a genome-wide association study has linked genes encoding members of IFN signaling pathways to critical illness in COVID-19 (149). However, Mendelian randomization revealed that life-threatening COVID-19 was associated with low expression of *IFNAR2*, but high expression of *TYK2* (149). Therapeutically, recombinant IFN β 1a did not reduce mortality in hospitalized patients with COVID-19 (150). In contrast, the anti-inflammatory therapy baricitinib, which has activity against JAK1/2 and moderate activity against TYK2, reduced mortality amongst hospitalized patients with COVID-19 by about 20%, and it has received U.S. Food and Drug Administration (FDA) approval for this indication (151). As discussed below, however, although JAK inhibitors may block IFN signaling, their effects are not limited to this pathway. In sum, the IFN response likely has a complex, bidirectional role in COVID-19, including the cardiac complications of COVID-19, both functioning in the host defence against viral infection, and contributing to the deleterious consequences of hyperinflammation in severe disease.

IFNs and atherosclerosis

Both type I IFNs and IFN- γ have also been implicated in the pathogenesis of atherosclerosis. In brief, type I IFNs may affect plaque formation through several different processes, including through the formation of foam cells and macrophage

extracellular traps, endothelial dysfunction and through influencing the actions of dendritic cells and T cells (152). IFN- γ affects cholesterol accumulation in macrophages and macrophage activation, induces foam cell formation and apoptosis, affects Th1-mediated immune responses, and promotes oxidative stress, endothelial activation, smooth muscle cell proliferation and plaque development (153, 154). Like its paradoxical actions in the heart, however, IFN- γ has been reported to have both pro- and anti-atherogenic effects (154). In this review, we have focused on the actions of IFNs in the heart and in heart disease. It should be noted that a comparably sized body of experimental evidence exists outlining the roles of both type I IFNs and IFN- γ in atherosclerosis. For an in-depth exposition, the reader is referred to reviews specifically on this topic (152–154).

IFNs as prognostic markers

Whereas IFNs and interferon-related pathways play several different roles in heart disease, evidence supporting circulating IFNs as biomarkers of cardiac disease is scant. Those data that do exist support a greater role for the measurement of plasma levels of IFN- γ than for type I IFNs. For instance, in a study of patients with HCM, increased plasma levels of IFN- γ were associated with LV wall thickness (155) (Figure 5). Furthermore, lower circulating levels of IFN- γ have been associated with greater likelihood of reverse remodeling following LVAD implantation (116), and of less severe peripartum cardiomyopathy (156) (Figure 5). For type I IFNs, plasma proteomic analysis revealed that higher circulating levels of IFNA5 were associated with a higher relative wall thickness in women, but not in men (157). It may be that determination of a type I IFN signature, as has been done in SLE for instance (42, 158), may offer greater prognostic value than measurement of individual IFNs. However, even amongst patients with SLE who often exhibit a strong type I IFN response, a type I IFN signature has not been robustly associated with CVD (44). In sum, extensive experimental evidence and correlative clinical studies support roles of IFNs and IFN-related pathways in the pathogenesis of heart disease (or protection against it). However, there is an absence of evidence that would suggest routine measurement of IFNs (or their downstream effectors) will offer utility as biomarkers of cardiac disease, certainly above already established measures, and even amongst at-risk groups.

Therapeutically targeting IFNs to improve outcomes in heart disease

To date, several different therapeutic approaches that augment or inhibit IFN pathways have received regulatory authority approval, although not for the treatment of heart disease. Most notably, these approaches include recombinant IFNs and small molecule JAK inhibitors. Elsewhere, there is fervent medicinal chemistry activity in the development of agents that interfere (or augment) cGAS-STING signaling, and newer strategies to modulate the actions of cytokines are under development.

Recombinant IFNs

As discussed earlier, recombinant IFN β 1b and recombinant IFN α 2b are approved for other indications, including multiple sclerosis, chronic hepatitis, and hematological malignancy. Whereas clinical studies have suggested improvements in some outcomes for patients treated with recombinant IFN in viral myocarditis (159–162), recombinant IFN therapy is not part of usual viral myocarditis management, with somewhat disappointing trial results attributed possibly to a relatively poor response to IFN of parvovirus B1 and HHV6 myocarditis (163). Similarly, as already discussed, recombinant interferon IFN β 1a did not affect mortality in hospitalized patients with COVID-19 (150).

JAK inhibitors

Whereas the goal of recombinant IFN therapy is to augment signaling through IFN pathways, JAK inhibitors block IFN signaling. These therapies have received regulatory approval principally for the treatment of inflammatory arthropathies, and they have shown promise in other disease settings associated with inflammation or an augmented IFN response. However, by virtue of blocking JAK/STAT signaling, the effects of these agents extend to antagonizing the actions of several proinflammatory cytokines whose receptors signal through this pathway and they are not limited to antagonizing IFNs or IFN-related pathways. Tofacitinib is a JAK1/3 inhibitor, with less efficacy vs. JAK2 and TYK2, and it is approved for the treatment of rheumatoid arthritis, psoriatic arthritis and ulcerative colitis (164). Baricitinib is an inhibitor of JAK1/2, with moderate activity vs. TYK2, with an indication for the treatment of rheumatoid arthritis (165). As already discussed, baricitinib has also received FDA approval for use in the treatment of COVID-19. Upadacitinib is selective for JAK1 and is approved for the treatment of rheumatoid arthritis, psoriatic arthropathy, axial spondyloarthritis, atopic dermatitis, ulcerative dermatitis and Crohn's disease (166). In a small clinical trial, baricitinib was shown to improve disease severity amongst patients with monogenic IFN-mediated autoinflammatory diseases [including chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperatures (CANDLE), SAVI, and other interferonopathies] (167). Baricitinib also reduced albuminuria and improved inflammatory markers in patients with diabetic kidney disease (168). However, the product monograph for baricitinib carries warnings as to a potential increased risk of infection, malignancy, major adverse cardiovascular events, and thrombosis (165). Accordingly, whereas JAK inhibitors offer a repurposing opportunity, their adverse effect profiles may preclude their use in the treatment of heart disease.

Strategies that antagonize cGAS-STING signaling

The development of inhibitors of cGAS or STING (70), or of TBK1 (169), is an area of active investigation from both the academic and the industrial sectors. Over 20 compounds have already been reported to have cGAS- or STING-inhibitory effects (70), and activators of the cGAS-STING pathway are being developed as cancer therapeutics (170, 171). cGAS inhibitors are being developed that exert their effects by either blocking the

catalytic site of cGAS (e.g., RU.521, Compound S3, G150, PF-06928125) or by interfering with cGAS binding to DNA (e.g., suramin, oligonucleotides, antimalarials) (70). Antagonists of STING have been developed that exert their effects by targeting the cGAMP binding site of STING (e.g., Astin C, tetrahydroisoquinolones) or by targeting STING palmitoylation which is necessary for its activation [e.g., indole ureas (especially H-151), nitrofurans, acrylamides, and nitro fatty acids] (70). Given the wealth of preclinical data attesting to the importance of cGAS-STING in the pathogenesis of cardiac disease, it will be interesting to see if any of these medicinal chemistry breakthroughs reach the clinical trial arena for this indication.

Future strategies to modulate signaling by IFNs and IFN-related pathways

Looking ahead, several different strategies are being trialed to either augment or antagonize cytokine action. The reader is directed to an excellent recent review on this topic (172). Briefly, various approaches are being developed to augment cytokine activity including the use of fusion proteins, PEGylation, polymeric matrices, microparticles, immune complexes and immunocytokines, orthogonal cytokines, mutagenesis, neokines, and surrogate agonists (172). Strategies to antagonize cytokine signaling that are under development include antibodies to cytokines or their receptors, JAK inhibitors, STAT inhibitors, proteolysis-targeting chimeras (PROTACs) and cytokine receptor targeting chimeras (or KineTACs) (172).

Summary and future directions

In this review, we have considered the actions of type I IFNs and ISGs, cGAS-STING pathway activation, IFN- γ , and IRFs in the heart. From the body of experimental evidence, it is clear that each of these biological processes plays important roles in either protection against heart disease or in the pathogenesis of diseases of the heart. How might it be possible to exploit these discoveries for the benefit of patients?

It is unlikely that measurement of plasma IFNs, certainly single IFNs, will offer prognostic value in the management of heart disease. Alternatively though, within the biomedical research space, recent years have witnessed enormous advances in single cell technologies. These single cell technologies, and in particular scRNA-seq, are especially helpful in defining immune cell subpopulations (173), and identification of immune cell subpopulations that are enriched for IFN-related genes has been important to several recent studies elucidating the molecular pathological basis of cardiac diseases (37, 102, 174–180). Fundamental studies exploiting single cell technologies and defining immune cell subpopulations based on IFN-related gene enrichment are likely to continue to advance our understanding of the role of inflammation in heart disease in years ahead.

Therapeutically, various strategies may be employed to dampen signaling by IFNs or IFN-related pathway signaling. However, immunosuppressive therapies may be associated with an increased risk of malignancy and infection. For prevalent diseases

such as heart disease, where therapies that improve outcomes already exist, the challenge will be the development of a therapeutic strategy that has an acceptable side effect profile. In this respect, the therapeutic targeting of a downstream pathway, such as cGAS-STING signaling, may offer theoretical advantages in that it leaves other immune defense pathways intact (70). Alternatively, a niche for therapies that antagonize IFN-related pathways may be found in the acute setting. Elsewhere, there has been renewed interest in the complex pathobiology of ISGs, and especially ISG15, since the emergence of SARS-CoV-2, and the recognition that ISG15 plays a key role in the defence against viral infection and in the immune response to viral infection (65). This renewed interest could pave the way for therapeutic advances for chronic diseases, such as heart disease, in the future. The biology of ISG15 is complex, the protein exerting different effects in its intracellular free or conjugated forms, and as a secreted protein, with species-specific differences between mice and humans (181). Similarly, it appears that IFN- γ can exert both beneficial and detrimental effects on the heart. However, just because the biology may be complicated, does not mean that it is not important.

In summary, IFNs and IFN-related pathways play important roles in the inflammation that commonly accompanies diseases of the heart. These roles are complex, being dependent on the nature of the underlying cardiac insult, the pathway itself, stage of disease, and host factors. As technological breakthroughs continue to advance the study of fundamental biology, a more nuanced understanding of the actions of IFNs and IFN-related pathway is sure to follow. Whether these advances will ultimately lead to improved outcomes for those affected by heart disease awaits to be seen.

Author contributions

DT: Writing – original draft, Writing – review & editing. SB: Writing – original draft, Writing – review & editing. AA: Writing – original draft, Writing – review & editing.

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Blood cell indices and inflammation-related markers with kidney cancer risk: a large-population prospective analysis in UK Biobank

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Background: Kidney cancer is a prevalent malignancy with an increasing incidence worldwide. Blood cell indices and inflammation-related markers have shown huge potential as biomarkers for predicting cancer incidences, but that is not clear in kidney cancer. Our study aims to investigate the correlations of blood cell indices and inflammation-related markers with kidney cancer risk.

Methods: We performed a population-based cohort prospective analysis using data from the UK Biobank. A total of 466,994 participants, free of kidney cancer at baseline, were included in the analysis. The hazard ratios (HRs) and 95% confidence intervals (CIs) for kidney cancer risk were calculated using Cox proportional hazards regression models. Restricted cubic spline models were used to investigate nonlinear longitudinal associations. Stratified analyses were used to identify high-risk populations. The results were validated through sensitivity analyses.

Results: During a mean follow-up of 12.4 years, 1,710 of 466,994 participants developed kidney cancer. The Cox regression models showed that 13 blood cell indices and four inflammation-related markers were associated with kidney cancer incidence. The restricted cubic spline models showed non-linear relationships with kidney cancer. Finally, combined with stratified and sensitivity analyses, we found that the mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelet distribution width (PDW), systemic immune-inflammation index (SII), and product of platelet count and neutrophil count (PPN) were related to enhanced kidney cancer risk with stable results.

Conclusion: Our findings identified that three blood cell indices (MCHC, RDW, and PDW) and two inflammation-related markers (SII and PPN) were independent risk factors for the incidence of kidney cancer. These indexes may serve as potential predictors for kidney cancer and aid in the development of targeted screening strategies for at-risk individuals.

KEYWORDS

kidney cancer, blood cell indices, inflammation-related markers, UK Biobank, prospective analysis

1 Introduction

As one of the most common urogenital malignancies, kidney cancer has garnered increased attention due to its rising incidence and significant mortality rates, accounting for 5% of all cancer cases and holding the sixth position among the most prevalent cancers in men (1–4). Kidney cancer, also known as renal cancer, is a disease that is becoming more common globally, with over 400,000 new cases reported each year. The mortality rate for kidney cancer is also high, with around 175,000 fatalities occurring annually worldwide (5–8). Despite its frequency, kidney cancer remains a complex and heterogeneous disease, characterized by challenges in early detection, limited treatment options, and an inadequate understanding of its underlying mechanisms (9). Several well-established risk factors have been identified, including age, sex, smoking, obesity, and hypertension (10, 11). However, numerous other factors that are suspected of elevating the risk of kidney cancer require further investigation (10).

Blood indices, such as the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and platelet count, have served as valuable indicators of hematological alterations that could potentially reflect systemic body state and tumor progression (12–15). These biomarkers provide valuable insights into the underlying pathophysiological processes and interactions within the tumor microenvironment (16, 17). Notably, kidney cancer patients frequently exhibit anemia, with up to 35% of cases demonstrating decreased levels of hemoglobin (HGB), hematocrit (HCT), MCV, and MCH due to the weak activity of erythropoietin (EPO) and abnormal iron metabolism (18, 19). For the red blood cell and platelet systems, preoperative reductions in HGB, HCT, MCV, and MCH have been linked to an increased predisposition for early recurrence and progression of kidney cancer (20, 21). Additionally, a decrease in the average size of platelets has emerged as an independent predictor of tumor-specific mortality (18). While these findings highlight the significance of abnormal hemograms in the progression and prognosis of kidney cancer, little attention is focused on the occurrence of kidney cancer (22, 23).

Inflammation plays an important role in the incidence and progression of cancer (24, 25). Systemic inflammation is usually

assessed through various biochemical or hematological markers routinely measured in common blood tests or as ratios from these measurements, which are called inflammation-related markers, including the systemic immune-inflammation index (SII), neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and product of platelet count and neutrophil count (PPN) (26–30). While increasing evidence indicates that these markers served as prognostic markers in newly diagnosed cancer patients with various malignancies, only a few studies have focused on the risk of cancer incidence (24, 31–33). As an immunogenic tumor, kidney cancer has a strongly evident interaction with inflammatory mediators (34). However, the association between inflammation-related markers and kidney cancer is still unclear.

In this paper, we aim to examine a wide range of blood cell indices and inflammation-related markers to determine their independent contributions to kidney cancer risk. Using a comprehensive prospective study design, we analyzed a large dataset comprising kidney cancer patients and matched controls, thereby providing valuable indexes into the development of this malignancy.

2 Methods

2.1 Study design

The UK Biobank is a large-scale prospective study designed to provide valuable resources for investigating the causes of various diseases. Comprehensive information regarding the study procedure and data collection can be accessed online or through relevant literature sources (<https://www.ukbiobank.ac.uk/media/gnkeyh2q/study-rationale.pdf>) (35). In brief, the study included participants who were registered with the NHS and lived within a 40-km radius of a UK Biobank assessment center. An initial invitation was extended to approximately 9.2 million individuals (36). Between 2006 and 2010, over 500,000 men and women aged between 40 and 69 years old consented to participate in the cohort and visited one of the 22 assessment centers located in England, Wales, and Scotland. The UK Biobank study was approved by the

National Health and Social Care Information Management Board and the North West Multicenter Research Ethics Committee (11/NW/0382). This approval ensures that the study was conducted in compliance with ethical guidelines and that the rights and privacy of the participants were protected.

2.2 Population selection

This study was performed following the guidelines and regulations outlined in UKB application number 61083. A total of 502,401 participants were included in our analysis. Initially, we excluded 32,677 participants who had missing blood cell indices. Subsequently, we eliminated 397 participants who had kidney cancer at baseline and were lacking critical covariate data ($N = 482$). Ultimately, the primary analysis consisted of 466,994 participants (Figure 1).

2.3 Blood cell indices

UKB comprises a comprehensive collection of blood cell indices extracted from biological samples. Using a hematology analyzer, exclusive count data were acquired, and other parameters were calculated accordingly (details at [https://biobank.ctsu.ox.ac.uk/](https://biobank.ctsu.ox.ac.uk/crystal/ukb/docs/haematology.pdf)

[crystal/ukb/docs/haematology.pdf](https://biobank.ctsu.ox.ac.uk/crystal/ukb/docs/haematology.pdf)). All indices included in this study were extracted from the initial visit and then classified as “red blood cell,” “immature red blood cell,” “white blood cell,” and “inflammation-related index.” The red blood cell category included RBC, HCT, MCV, MCH, MCHC, and RDW. The immature RBC category included the reticulocyte count, reticulocyte percentage, nucleated RBC, nucleated RBC percentage, MRV, MSCV, and IRF. The white blood cell category included WBC, basophil count (BASO), basophil percentage, eosinophil count (EO), eosinophil percentage, monocyte count, monocyte percentage, neutrophil count (NEUT), neutrophil percentage, lymphocyte count (LYMPH), lymphocyte percentage, MPV, platelet distribution width, and platelet (PLT) and high light scatter reticulocyte percentage. For a more complete examination of the connection between “inflammation-related marker” and the incidence of kidney cancer, SII, PLR, NLR, and PPN were calculated. PLT, NEUT, and LYMPH concentrations were measured at 1,000 cells/ μ L. PLT was multiplied by (NEUT/LYMPH) to arrive at SII. The PLR was computed by dividing the PLT by the LYMPH. NLR was determined using NEUT/LYMPH. PPN was determined by multiplying PLT by NEUT. All analytes within the dataset had identical detection limits. None of the results fell below the threshold for detection. [Supplementary Table S1](#) of the [Supplementary Materials](#) shows a list of indicators with their corresponding abbreviations and data fields.

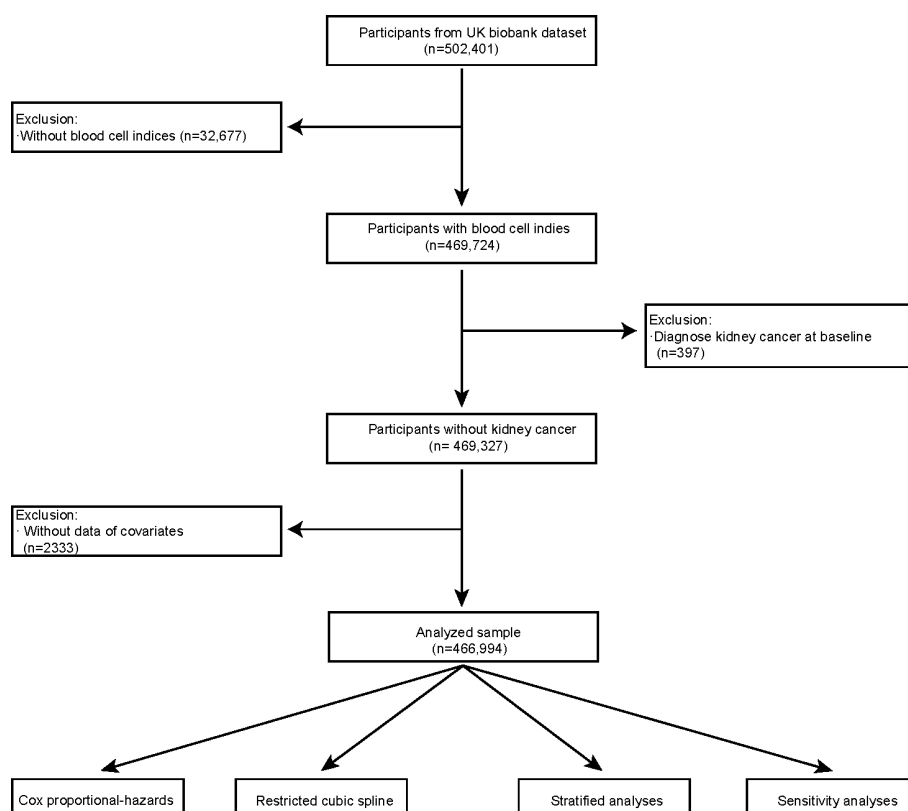


FIGURE 1
Flowchart of the research design.

2.4 Covariates

We utilized the baseline touch-screen questionnaire to evaluate a number of potential confounding variables: age (years), sex, race (white, mixed, Asian, black, etc.), household income, lifestyle behavior (smoking status and alcohol drinker status), and body mass index (BMI). The questionnaire yielded the codes a degree (college or university degree) or no degree for education. The Townsend Deprivation Index is a composite measure of deprivation based on nonhome ownership, non-car ownership, unemployment, and domestic overcrowding that indicates the socioeconomic status of the participant. Adapted from the American Heart Association Guidelines, a balanced diet score was defined as adhering to four or five of the following components: (1) total fruit consumption of 4.5 pieces per week, (2) total vegetable consumption of 4.5 servings per week (three tablespoons of vegetable considered as one serving), (3) total fish consumption of two servings per week, (4) processed meat consumption at two times per week, and (5) red meat consumption at five times per week. The comorbidity variables included hypertension (Field 6150) and diabetes (Field 2443).

2.5 Kidney cancer diagnosis

This study's results were C64 (kidney malignant tumor, excluding renal pelvis). The International Classification of Diseases (ICD) classification system was utilized to record the diagnoses. The incident disease in this study was determined by the primary or secondary diagnoses from hospital admission data or by the primary or secondary causes after the baseline data collection. The participants were observed from the time of baseline until the date of the first diagnosis or on December 31, 2021.

2.6 Statistical analysis

The mean (standard deviation, SD) and number (percentage) for continuous variables were obtained. Figure 1 illustrates the study design flowchart. Blood cell indices were log-transformed and normalized to Z scores [$Z = (\text{value} - \text{mean})/\text{SD}$] prior to Cox analysis so that the hazard ratio (HR) represents the effect per SD increment. Model 1 represented the unadjusted model, which did not consider any covariates. Model 2, the minimally adjusted model, accounted for gender and age as potential confounding variables. Model 3, the fully adjusted model, considered a comprehensive set of covariates including sex, age, race, qualification, BMI, smoking, alcohol consumption, health diet score, household income, diabetes, and hypertension. The potential for nonlinearity was investigated using restricted cubic spline models fitted to Cox models and adjusted for covariates as in model 3. Potential nonlinearity was examined by comparing the linear model to the model containing both linear and cubic spline terms using the likelihood ratio test. Then, we performed stratified analyses to estimate the potential modification effects according to

age (≤ 60 and > 60 years), sex (male or female), race (white, mixed, Asian, black, and others), smoking status (current, former, and never), alcohol drinker status (current, former, and never), qualifications (with college or university degree or none), diabetes (yes and no), hypertension (yes and no), and BMI (≤ 25 and > 25). By modeling the cross-product terms of the stratifying variables with each blood cell index, interaction *P* values were examined. In the sensitivity analysis, we first applied models 1 and 2 to test the robustness of our findings. Second, we used quantile regression for further analysis. Third, we used the first repeat assessment visit of blood cell index data in 2012 and conducted Cox regression analysis to support the stability of the results.

All statistical analyses were carried out using R version 4.2.1. All *P* values listed below were adjusted.

3 Results

3.1 Baseline characteristics

Baseline characteristics stratified by kidney cancer status are shown in Table 1. A total of 466,994 kidney cancer-free participants from UKB were included in the primary analyses. Overall, the mean age of the participants was 56.5 (± 8.1) years; 214,089 (45.8%) were male patients and 252,905 (54.2%) were female patients. During a mean follow-up time of 12.4 years, 1,710 participants developed kidney cancer. Among them, 1,094 (64.0%) participants were male patients, and 616 (36.0%) participants were female patients.

3.2 Blood cell indices and kidney cancer risk

In the RBC category, decreased levels of MCV [HR 0.90, 95% confidence interval (CI): 0.86–0.95, $P < 0.0001$] and MCHC (HR 0.91, 95% CI: 0.876–0.96, $P < 0.0006$), indicative of an anemia state, were associated with higher kidney cancer incidence in the fully adjusted models (model 3). RDW, indicative of the heterogeneity of erythrocytes, was positively associated with kidney cancer risk (HR 1.10, 95% CI: 1.05–1.14, $P < 0.0001$). U-shaped relationships were discovered between RBC (P for nonlinearity = 0.0007; Figure 2), HCT (P for nonlinearity < 0.0002 ; Figure 2), MCV (P for nonlinearity = 0.0227; Figure 2), and MCHC (P for nonlinearity = 0.0115; Figure 2) with incident kidney cancer.

In the immature RBC category, decreased reticulocyte maturation parameters, including NRBC (HR 0.88, 95% CI: 0.78–0.99, $P = 0.0391$), NRBC% (HR 0.88, 95% CI: 0.78–0.99, $P = 0.0391$), MRV (HR 0.94, 95% CI: 0.90–0.99, $P = 0.0180$), and MSCV (HR 0.88, 95% CI: 0.83–0.92, $P < 0.0001$), were significantly associated with a higher kidney cancer risk. MRV (P for nonlinearity = 0.00051) had a U-shaped association with kidney cancer risk. MSCV had a linear association with kidney cancer risk, rapidly reaching the lowest risk at ≈ 80 and becoming flat thereafter.

In the WBC category, increased levels of WBC (HR 1.04, 95% CI: 1.03–1.05, $P < 0.0001$), NEUT (HR 1.14, 95% CI: 1.11–1.17, $P <$

TABLE 1 Baseline characteristics of UK Biobank participants by incident kidney cancer incidence.

Characteristics	Overall (N = 466,994)	No kidney cancer (N = 465,284)	Kidney cancer (N = 1,710)	P value
Mean follow-up duration (years) (SD)	12.4 ± 1.0	12.4 ± 0.9	6.5 ± 3.5	<0.001
Age, mean (SD), years	56.5 ± 8.1	56.5 ± 8.1	60.6 ± 6.5	<0.001
Gender (%)				<0.001
Male	214 089 (45.8)	212,995 (45.8)	1,094 (64.0)	
Female	252,905 (54.2)	252,289 (54.2)	616 (36.0)	
Race (%)				<0.001
White European	44,1207 (94.5)	439,551 (94.5)	1,656 (96.8)	
Mixed	2,717 (0.6)	2,711 (0.6)	6 (0.4)	
South Asian	8,798 (1.9)	8,777 (1.9)	21 (1.2)	
Black	7,073 (1.5)	7,061 (1.5)	12 (0.7)	
Others	7,199 (1.5)	7,184 (1.5)	15 (0.9)	
With college or university degree (%)	151,108 (32.4)	150,686 (32.4)	422 (24.7)	<0.001
BMI, mean (SD), kg/m²	27.4 ± 4.8	27.4 ± 4.8	28.9 ± 4.9	<0.001
Smoking status (%)				<0.001
Current	48,996 (10.5)	48,757 (10.5)	239 (14.0)	
Former	161,651 (34.6)	160,930 (34.6)	721 (42.2)	
Never	254,506 (54.5)	253,763 (54.5)	743 (43.5)	
Missing	1,841 (0.4)	1,834 (0.4)	7 (0.4)	
Alcohol drinker status (%)				0.001
Current	429,274 (91.9)	427,711 (91.9)	1,563 (91.4)	
Former	16,682 (3.6)	16,604 (3.6)	78 (4.6)	
Never	20,387 (4.4)	20,325 (4.4)	62 (3.6)	
Missing	651 (0.1)	644 (0.1)	7 (0.4)	
Health diet score, mean (SD)	2.2 ± 0.9	2.2 ± 0.9	2.1 ± 0.9	<0.001
Household income (%)				<0.001
<18,000 (£)	19,707 (4.2)	19,638 (4.2)	69 (4.0)	
18,000–30,999 (£)	138,248 (29.6)	137,642 (29.6)	606 (35.4)	
31,000–51,999 (£)	101,721 (21.8)	101,297 (21.8)	424 (24.8)	
52,000–100,000 (£)	104,384 (22.4)	104,039 (22.4)	345 (20.2)	
>100,000 (£)	81,350 (17.4)	81,125 (17.4)	225 (13.2)	
“Do not know” or missing	21,584 (4.6)	21,543 (4.6)	41 (2.4)	
Health status (%)				
Diabetes history	24,210 (5.18%)	24,053 (5.17%)	157 (9.18%)	<0.001
Hypertension	111,708 (23.92%)	111,055 (23.87%)	653 (38.19%)	<0.001

For continuous variables, data are presented as mean (SD), and for categorical variables, data are presented as number (percentage). The level of education was classified as either higher (college/university degree) or lower.

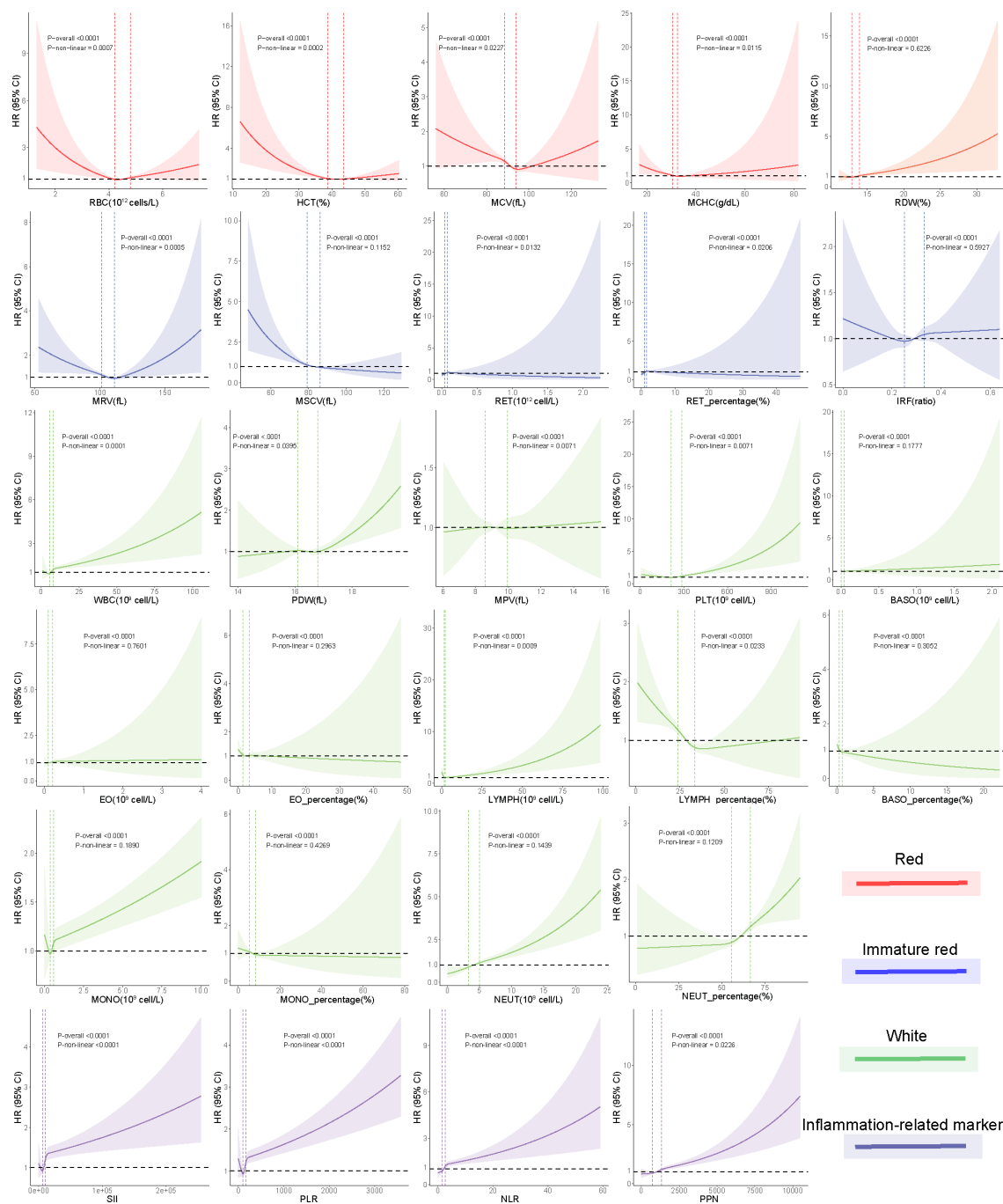


FIGURE 2

Nonlinear associations between blood cell indices and risk of incident kidney cancer using restricted cubic spline models. Two vertical dashed lines represent the 25% and 75% exposure values for each exposure. Adjustments were made for demographic and socioeconomic factors. The red, green, blue, and purple hues denote that each blood cell measurement corresponds to the "red blood cell," "immature red blood cell," "white blood cell," and inflammation-related index categories, respectively.

0.0001), and NEUT% (HR 1.17, 95% CI: 1.12–1.23, $P < 0.0001$) trended toward a higher kidney cancer risk, while increased levels of LYMPH% (HR 0.85, 95% CI: 0.81–0.89, $P < 0.0001$) showed protective effects against incident kidney cancer. Among platelet indices, we found

significant associations between kidney cancer incidence and PDW (HR 1.07, 95% CI: 1.02–1.12, $P = 0.0054$) and PLT (HR 1.12, 95% CI: 1.09–1.15, $P < 0.001$). U-shaped associations were also found between PLT (P for nonlinearity < 0.0001 ; Figure 2) and kidney cancer risk.

3.3 Inflammation-related markers and kidney cancer risk

We found that white blood indices were significantly positively associated with the incidence of kidney cancer. Thus, we hypothesize that inflammation status may be associated with kidney cancer. We developed four inflammation-related markers, including the systemic immune-inflammation index (SII), platelet-to-lymphocyte ratio (PLR), neutrophil-to-lymphocyte ratio (NLR), and the product of platelet count and neutrophil count (PPN). In the inflammation-related index category, increased SII (HR 1.02, 95% CI: 1.01–1.03, $P < 0.0001$), PLR (HR 1.02, 95% CI: 1.01–1.03, $P < 0.0001$), NLR (HR 1.04, 95% CI: 1.02–1.05, $P < 0.0001$), and PPN (HR 1.13, 95% CI: 1.11–1.16, $P < 0.0001$) trended toward a higher kidney cancer risk (Figure 3). U-shaped associations were also found between PLT (P for nonlinearity <0.0001 ; Figure 2) and kidney cancer risk. RCS models revealed a positive association between the inflammation-related index and kidney cancer risk (Figure 2). Then, we conducted

quantile regression between the inflammation-related markers and incident kidney cancer. We divided the inflammation-related index into four quartiles (Q1–Q4) (Table 2). We found that Q4 had a higher incidence of kidney cancer in all models, while P for trend was significantly different in model 3. In Q4, SII (HR 1.47, 95% CI: 1.29–1.68, $P < 0.0001$), PLR (HR 1.44, 95% CI: 1.26–1.64, $P < 0.0001$), NLR (HR 1.57, 95% CI: 1.36–1.81, $P < 0.0001$), and PPN (HR 1.55, 95% CI: 1.35–1.78, $P < 0.0001$) trended toward a higher kidney cancer risk compared with Q1 in model 3 (Table 2).

3.4 Stratified analyses

Based on possible effect modifiers and blood cell indices risk variables, HRs in stratified analyses were often in the same direction. In the RBC category, we found that the associations of MCV, MCHC, and RDW were stronger among older, male, white European, former smoker, current alcohol drinker, people with

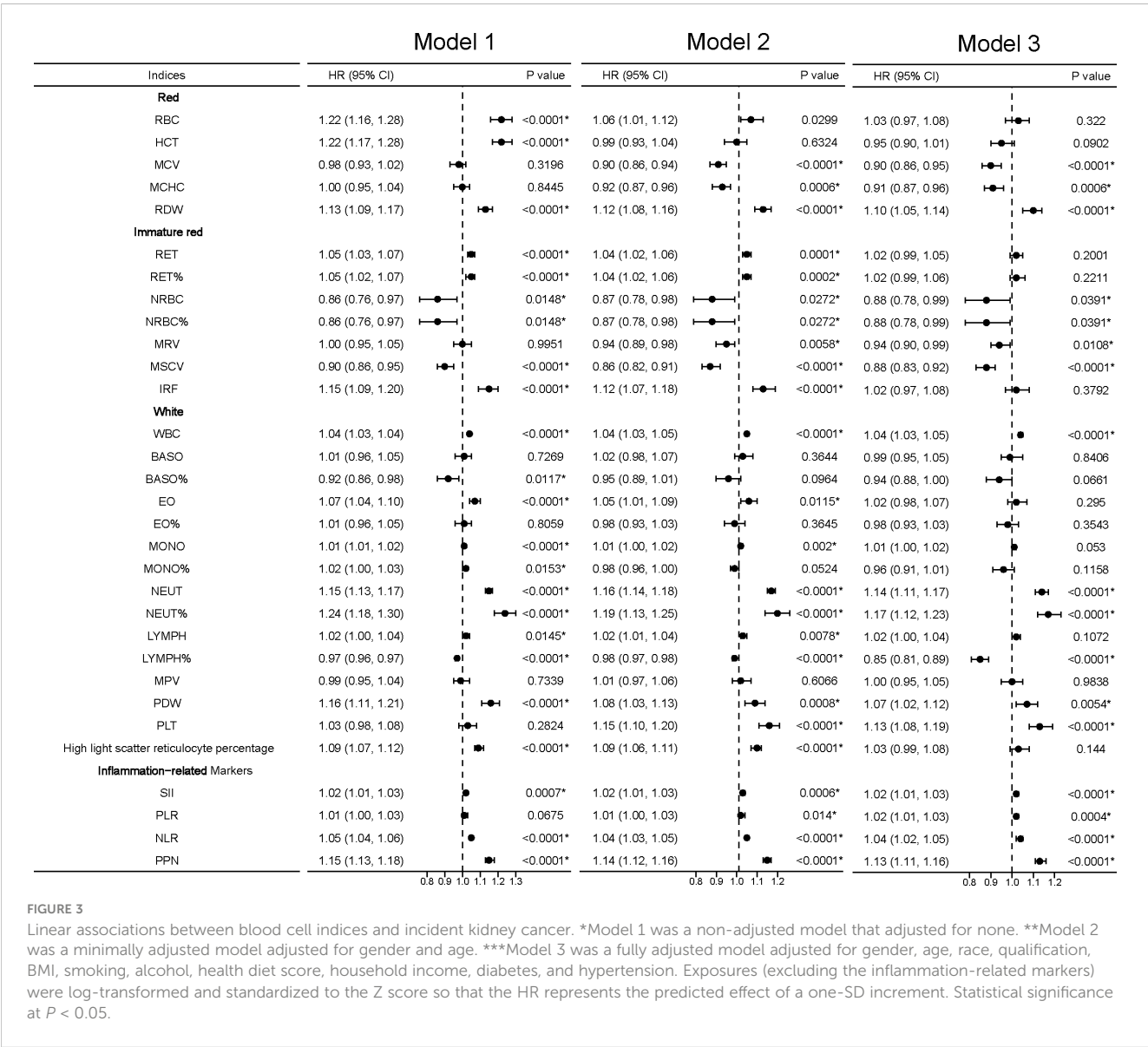


TABLE 2 Quantile regression between inflammation-related markers and incident kidney cancer.

Inflammation-related index	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Model 3 HR (95% CI)
Systemic immune-inflammation index (SII)			
Q1	Reference		
Q2	0.97 (0.85, 1.12)	1.02 (0.89, 1.17)	1.06 (0.92, 1.22)
Q3	0.94 (0.82, 1.08)	1.01 (0.88, 1.16)	1.08 (0.93, 1.24)
Q4	1.26 (1.11, 1.43)	1.33 (1.17, 1.52)	1.47 (1.29, 1.68)
P trend	<0.001	<0.001	<0.001
Platelet-to-lymphocyte ratio (PLR)			
Q1	Reference		
Q2	0.96 (0.84, 1.10)	1.03 (0.90, 1.18)	1.10 (0.96, 1.26)
Q3	0.93 (0.81, 1.07)	1.03 (0.90, 1.18)	1.14 (0.99, 1.31)
Q4	1.13 (0.99, 1.29)	1.25 (1.10, 1.43)	1.44 (1.26, 1.64)
P trend	0.068	<0.001	<0.001
Neutrophil-to-lymphocyte ratio (NLR)			
Q1	Reference		
Q2	1.22 (1.05, 1.42)	1.17 (1.00, 1.36)	1.14 (0.98, 1.33)
Q3	1.46 (1.26, 1.69)	1.34 (1.16, 1.55)	1.30 (1.12, 1.51)
Q4	1.92 (1.67, 2.20)	1.61 (1.40, 1.86)	1.57 (1.36, 1.81)
P trend	<0.001	<0.001	<0.001
Product of platelet count and neutrophil count (PPN)			
Q1	Reference		
Q2	1.12 (0.96, 1.30)	1.13 (0.98, 1.31)	1.08 (0.93, 1.25)
Q3	1.34 (1.17, 1.55)	1.40 (1.22, 1.61)	1.29 (1.12, 1.48)
Q4	1.62 (1.41, 1.86)	1.80 (1.57, 2.06)	1.55 (1.35, 1.78)
P trend	<0.001	<0.001	<0.001

Model 1 was a non-adjusted model that adjusted for none. Model 2 was a minimally adjusted model adjusted for gender and age. Model 3 was a fully adjusted model adjusted for gender, age, race, qualification, BMI, smoking, alcohol, health diet score, household income, diabetes, and hypertension.

college or university degree, no diabetes, hypertension, and BMI >25 participants. In the immature red category, MSCV and MRV showed significant associations among the above-mentioned specific groups, while NRBC and IRF were not remarkable. In the WBC category, we found that associations of WBC, NEUT, NEUT%, LYMPH%, and PDW had similar trends in both age

and gender, while PLT had stronger associations in the older participants. For the inflammation-related index category, SII, PLR, NLR, and PPN had similar results to the RBC category (Supplementary Tables S2–S5).

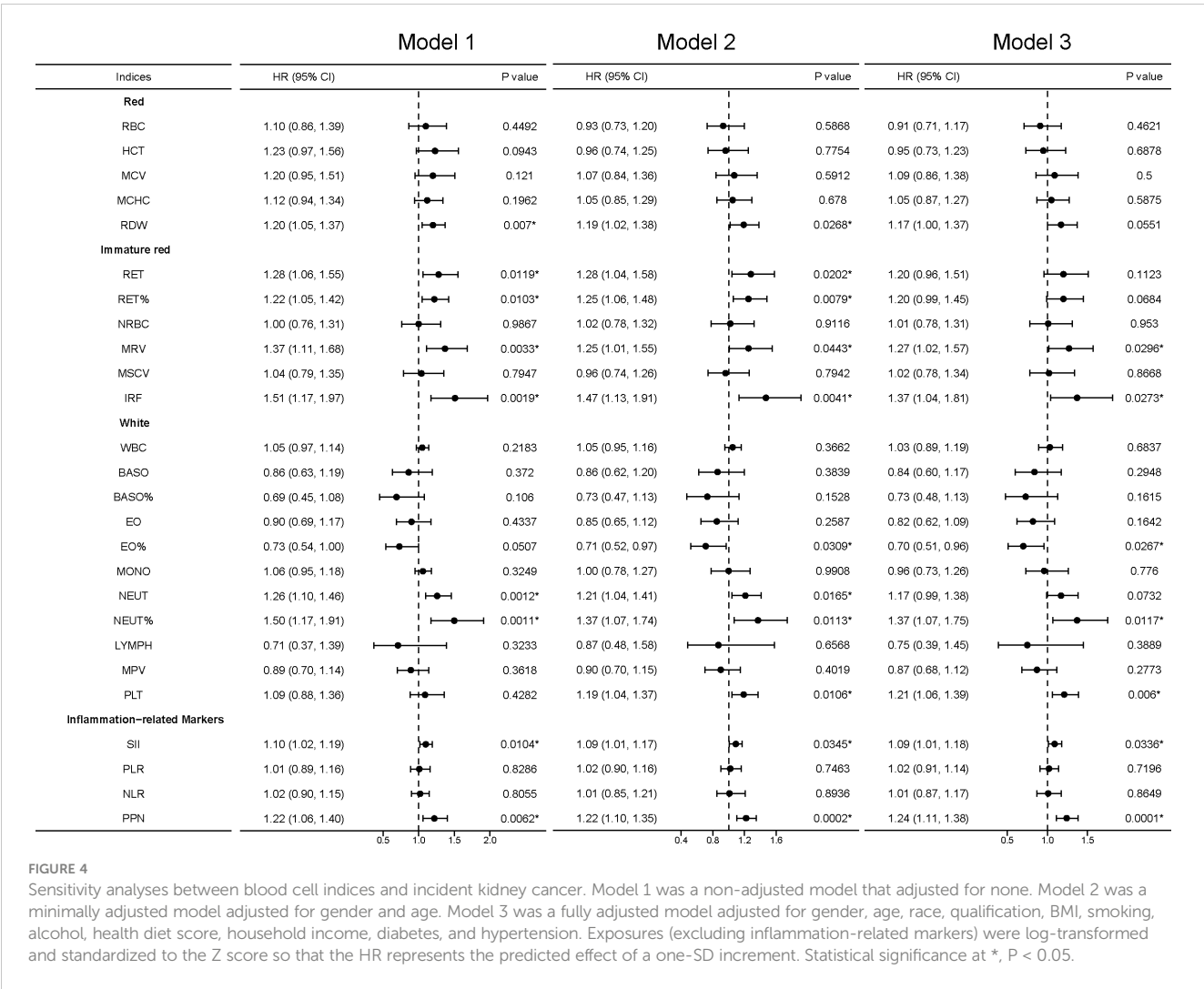
3.5 Sensitivity analyses

In sensitivity analyses, we found that the association between the RBC category and kidney cancer risk lost significance in model 3, including MCV, MCHC, and RDW, while the association between RDW and kidney cancer risk remained significant in model 1 and model 2. For the immature RBC category, MRV maintained a significant difference and IRF regained a significant difference in model 3. For the WBC category, EO%, NEUT%, and PLT were significantly associated with kidney cancer risk. The inflammation-related markers SII and PPN had a stable association (Figure 4).

4 Discussion

To the best of our knowledge, this is a comprehensive prospective investigation of the connection between 27 blood cell indices and incident kidney cancer. The study utilized data from a substantial cohort of 466,994 participants from the UK Biobank, with an average follow-up period of 12.4 years. We confirmed that three blood cell indices (MCHC, RDW, and PDW) and two inflammation-related markers (SII and PPN) were associated with the risk of kidney cancer. However, further investigation is needed to elucidate the underlying mechanisms responsible for these associations. In the future, we can predict tumor risk by building disease prediction models based on blood cells. At the same time, it provides another strategy for population screening, and further detection of outliers in the blood cell index population could exclude the risk of tumor occurrence. Finally, our research can guide the direction of future basic research. Various exposure factors may promote the development or progression of tumors by mediating the circulation of blood cells.

The detection, classification, and function of circulating immune cells are helpful to further understand the complex mechanism of tumors. The changes in hematological indexes reflect the changes of the internal environment in the body, which may provide necessary conditions for the occurrence and development of tumors. Hypoxia plays an important role in cancer progression. Dynamin-related protein 1 (Drp1) was an important protein that controls the quality of mitochondria and cellular processes through alterations in its oligomeric structure and other modifications that may be associated with hematological index changes (37). Single-cell RNA sequencing could provide a new insight into the tumor immune microenvironment (38, 39). Emerging nano-/biotechnology could drive oncolytic virus-activated and combined cancer immunotherapy through hematological mediation, and some supramolecular biomaterials could also be applied for cancer immunotherapy (40–43). At present, more and more detection techniques based on multi-



omics have played an important role in the diagnosis, treatment, and identification of tumors. Our study highlights the possible role of immune-associated cells in the development of kidney cancer.

It is widely reported that MCHC plays an important role in regulating the development and progression of several cancers, such as non-small cell lung cancer (44), prostate cancer (45), oral squamous cell carcinoma, and head and neck cancers (46). We found that a decreased level of MCHC was significantly correlated with a higher kidney cancer incidence at first. MCHC is an important index of anemia, and anemia is hypothesized to be an independent adverse prognostic indicator in patients with kidney cancer (20, 47). Behind anemia, the accompanying hypoxia may play a critical role. Hypoxia, a condition in which tissues are oxygen-deprived, upregulates the expression of hypoxia-inducible factor (HIF), which then induces hundreds of genes in an HIF-dependent manner to encode proteins that play key roles in numerous aspects of cancer biology, such as proliferation, cell survival, epithelial-to-mesenchymal transition (EMT), angiogenesis, invasion, and metastasis (48). Consequently, we infer that MCHC impacts kidney cancer risk, possibly through anemia and HIF pathways (49).

RDW, which reflects erythrocyte volume and size heterogeneity, is regarded as a strong and independent risk factor for death in the general population and is expected to be an effective indicator (50). In addition, it is generally recognized that RDW is a risk factor for various diseases, such as cardiovascular disease, venous thromboembolism, diabetes, and so on (50). Actually, we found that RDW was positively associated with kidney cancer risk, which supports RDW as a potential diagnosis marker for kidney cancer.

Platelets have been reported to influence cancer progression, metastasis, and angiogenesis in multiple ways, such as protecting tumor cells from high shear forces in the blood circulation and leukocyte attack and binding to C-type lectin-like receptor 2 (CLEC-2) to facilitate hematogenous cancer metastasis and cancer-associated thrombosis (12, 51). It is well documented that a higher platelet count is linked with shorter disease-specific survival in kidney cancer (52). PDW reflects the variation of platelet volume in the blood. The results demonstrated that elevated PDW is linked with a high kidney cancer incidence, which demonstrated that PDW might be an effective risk factor for kidney cancer.

While previous research discovered that the inflammation marker SII was connected with kidney cancer risk (53), the

association of PPN and kidney cancer has not been clear. Our paper showed that both SII and PPN were related to kidney cancer risk. Inflammatory conditions can exist in some types of cancer prior to the development of a malignant transformation. In other types of cancer, an oncogenic alteration causes an inflammatory microenvironment which improves cancer cell growth (54, 55). However, inflammatory conditions can exist in some types of cancer prior to the development to a malignant transformation, and chronic inflammation is generally acknowledged as an independent risk factor for the development of a majority of cancers (56). Another study suggests that inflammatory pathways can promote kidney cancer cell growth and immune evasion (34), which may provide theoretical bases for inflammation markers acting as risk factors for kidney cancer.

Several studies have shown a strong association between SII and kidney cancer. A study showed that SIRI and SII indexes show a moderate efficiency to show metastases in RCC (57). A large multi-center longitudinal study showed that SII increases the risk of total and cause-specific mortality among patients with chronic kidney disease, including kidney cancer (58). SII could also predict the survival of patients with renal cell cancer treated with nivolumab (59). Now, most articles have studied the relationship between SII and renal cancer progression and response to treatment. However, the incidence of kidney cancer and inflammation-related markers still requires further research.

There are several advantages to this study. First, this is a comprehensive examination of the association between blood indices and incident renal cancer. Second, owing to the large sample size, lengthy follow-up period, and extensive measurement of covariates, our study is longitudinal and possesses distinct advantages. Third, we summarized the mechanisms underpinning the association between blood indices and kidney cancer, which provides strong evidence for clinical application.

There are inevitably some limitations and deficiencies. Because these blood indices may be associated with health status and are susceptible to other factors such as comorbidities, socioeconomic status, and poor health, it is not feasible for this analysis to rule out detection bias. Then, the identification of UKB is determined by the ICD classification. Regrettably, the ICD categorization system relies on morphology rather than pathology, making it impossible to differentiate the relative risk of various blood cells for different forms of kidney cancer based on their pathological characteristics. In addition, the majority of UKB participants are European, which necessitated the use of stratified analyses that limited the generalizability of our results.

Additional limitations can be due to the fact that the pathogenesis of renal cancer is still unclear, and many factors influence blood cell indices. Hypertension and diabetes were the main risk factors of kidney cancer that could affect blood cell indices. Cigarette smoking and obesity are also likely to affect kidney cancer incidence trends and also included in the covariates (60–62). However, inadequate adjustment of covariates may lead to a bias in this study. Thus, establishing a larger cohort associated with renal cancer will help further explore the role of circulating immune cells. The simultaneous use of high-throughput

sequencing and more detailed case data could also help further understand the specific mechanisms of kidney cancer progression. Establishing a kidney cancer-related cohort based on a diverse population is necessary to improve generalizability.

5 Conclusion

This study identified three blood cell indices and two inflammation-related markers as dependent risk factors for kidney cancer incidence. As these indexes could be obtained through routine blood tests, they would be useful in large-scale screening or primary care settings to help discover individuals who might benefit from early screening or targeted prevention strategies for kidney cancer. Additional research is necessary to further illustrate the underlying mechanisms by which these blood cell indices and inflammation-related markers are associated with kidney cancer risk.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#). Further inquiries can be directed to the corresponding authors.

Ethics statement

This research was conducted under the UK Biobank application 61083. The UK Biobank study was approved by the National Health and Social Care Information Management Board and the North West Multicenter Research Ethics Committee (11/NW/0382). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

QH: Conceptualization, Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. CW: Conceptualization, Data curation, Formal analysis, Methodology, Writing – review & editing. LC: Validation, Writing – review & editing. PZ: Validation, Writing – review & editing. WZ: Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. FC: Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fonc.2024.1366449/full#supplementary-material>

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