Original Article

Association between reactive oxygen species production in neutrophils and liver fibrosis in the general population

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Fibrosis, induced by reactive oxygen species (ROS) production in neutrophils, has harmful effects on the liver and various other organs. However, little is known about the association between liver fibrosis and ROS levels in neutrophils in the general population. This large-scale epidemiological study aimed to determine the association between liver fibrosis and neutrophil-generated ROS levels according to age and sex in the general population. This cross-sectional study included 1,000 participants from a district health promotion project. Participants were grouped based on sex (male; female) and age (young, <65 years; old, ≥65 years). The four groups were as follows: male, young (*n* = 289); male, old (*n* = 100); female, young (*n* = 425); and female, old (*n* = 186). Liver fibrosis was assessed using the fibrosis 4 (FIB-4) index, aspartate aminotransferase-to-platelet ratio index (APRI), and non-alcoholic fatty liver disease (NAFLD) fibrosis score (NFS). Basal and stimulated ROS were considered in the analysis. Multiple linear analyses showed (1) significant positive correlations between all liver fibrosis scores and basal ROS in the young groups, and (2) significant negative correlations between NFS and stimulated ROS in females. Preventing liver fibrosis through neutrophil-related immune system enhancement may avert the development of lifestyle-related diseases and infections.

***Key Words*:** **liver fibrosis, FIB-4 index, aspartate aminotransferase to platelet ratio index, NAFLD fibrosis score, reactive oxygen species**

Advanced stages of alcoholic liver disease, hepatitis B and C, and non-alcoholic fatty liver disease (NAFLD) can all lead to liver fibrosis. Advanced liver fibrosis progresses to cirrhosis and is a risk factor for liver cancer. Moreover, the degree of fibrosis is strongly related to prognosis, including overall mortality, in many liver diseases.[1,2] Evidence also suggests that NAFLD is a contributing factor to metabolic syndromes and cardiovascular events associated with the development of liver fibrosis.[3,4] Thus, liver fibrosis has diverse and harmful effects not only on the liver, but also on various organs.

Reactive oxygen species (ROS) are constantly produced in the body, and many ROS are produced in the liver, where metabolic activity is high. The liver produces ROS such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\bullet}OH$). The balance between ROS production and elimination is maintained by ROS-scavenging enzymes such as superoxide dismutase (SOD) and catalase. The state in which the amount of ROS produced is greater than that eliminated by the scavenging system is called oxidative stress and is considered to be one of the etiologies of chronic liver disease. Excessive oxidative stress causes liver fibrosis through the production of inflammatory cytokines.[5,6] In a vicious cycle, the consequent development of liver fibrosis further increases oxidative stress. Alcohol consumption, hepatitis B and C, NAFLD, and many other chronic liver diseases increase oxidative stress through iron overloading, increased cytokine levels, and endotoxemia.[7–12] Chronic liver disease leads to cirrhosis via liver fibrosis. Drugs with antioxidant mechanisms have been reported to be useful in the treatment of liver disease, and vitamin E, which has antioxidant properties, is used for non-alcoholic steatohepatitis (NASH) in clinical medicine.[13]

In the liver, infiltrating Kupffer cells and neutrophils also produce ROS that are activated by the immune system.[14] Neutrophils produce ROS through nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. The binding of antibodies and immune complexes to Fc and C3b receptors on the surface of neutrophils activates NADPH oxidase and produces $O_2^{\bullet-}$. Superoxide is converted to hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\bullet}OH$) by myeloperoxidase (MPO). Neutrophils play a central role in innate immunity and are the first line of defense against foreign substances, including microorganisms. Neutrophils engulf microorganisms and produce ROS [stimulated ROS (SROS) production] in the process.[15] Still, neutrophils continuously produce ROS, even when they are not stimulated (basal ROS production). Although ROS in neutrophils have beneficial effects, such as the removal of foreign substances, excessive ROS production is directly or indirectly involved in a wide variety of clinical disorders, such as atherosclerosis, pulmonary toxicity, and cancer.[16,17]

An association between liver fibrosis and atherosclerosis has been reported in NAFLD, with the NAFLD/NASH guidelines recommending a detailed examination of cardiocerebrovascular diseases in the liver fibrosis group.[18] Among the common pathological factors associated with the development of liver fibrosis and atherosclerosis, oxidative stress in the context of metabolic syndrome, obesity, glucose intolerance, and lipid abnormalities has been documented.[19–21] ROS in neutrophils has a major influence on the development and destabilization of atherosclerotic plaques.[22] In addition, ROS in liver-derived neutrophils is involved in atherosclerotic plaque formation in NASH.[22] Therefore, the development of liver fibrosis increases ROS levels in neutrophils, which may cause not only liver damage, but also clinical disorders in various organs, such as atherosclerosis.

Although fundamental studies on the association between

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liver fibrosis and neutrophil-generated ROS have been widely conducted, epidemiological studies on this relationship in the general population are lacking. A major reason for the lack of epidemiological studies on the general population is the need for invasive biopsies to diagnose liver fibrosis. However, scoring systems employing age, body size, and blood test values have been developed, making it possible to noninvasively assess liver fibrosis during health checkups. Among the many scoring systems, the fibrosis 4 (FIB-4) index, aspartate aminotransferase-to-platelet ratio index (APRI), and NAFLD fibrosis score (NFS) are useful. The FIB-4 index and APRI were developed as evaluation tools for hepatitis C while the NFS was created for NAFLD but these indices can be calculated from data collected during general health checkups.[23–25] Using the aforementioned scoring systems that take into account sex and age, this study aimed to investigate the association between neutrophil-derived ROS and liver fibrosis in the general population through a large epidemiological study.

Materials and Methods

Study participants. In total, 1,113 adult participants were recruited from the Iwaki Health Promotion Project conducted in June 2015 in the Iwaki District of Hirosaki City, Northern Japan (Fig. 1). Of these, 113 who had a history of malignant tumors or liver disease, were taking steroids or antibiotics, and had missing data were excluded from the study.

Clinical parameters. The following clinical parameters were recorded on the same day: sex, age, body mass index (BMI, calculated by dividing the weight in kilograms by the squared height in meters), and blood levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), albumin, total bilirubin, glucose, insulin, white blood cells (WBC), Neutrophils, hemoglobin A1c (HbA1c), and platelets. Medical history and medication status were investigated using questionnaires and interviews.

Scoring of liver fibrosis. The FIB-4 index, APRI, and NFS were used as indicators of liver fibrosis. The scoring formulas were as follows:

FIB-4 index: [age (years) × AST (U/L)]/[platelet count ($\times 10^9$/L) × ALT (U/L)].

APRI: [AST (U/L)/upper limit of normal × 100]/platelet count ($\times 10^9$/L).

NFS: $-1.675 + 0.037 \times$ age (years) $+ 0.094 \times$ BMI (kg/m^2) + 1.13 × diabetes or impaired glucose tolerance (yes = 1, no = 0) + 0.99 × AST (U/L)/ALT (U/L) − 0.013 × platelet (10^9/L) − 0.66 × albumin (g/dl)

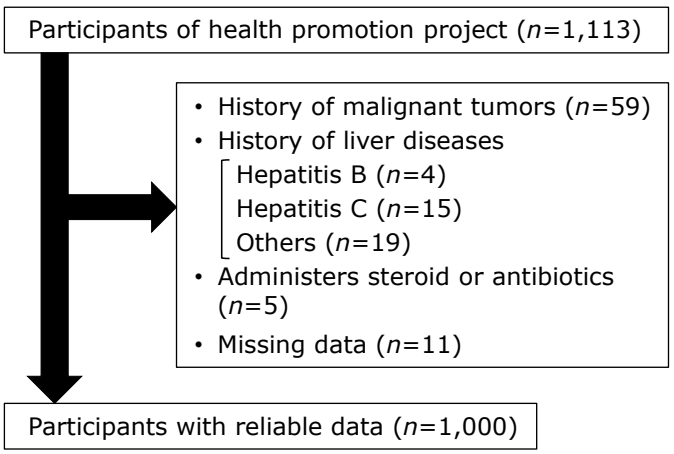

Fig. 1. Flowchart of study participants.

Neutrophil functions. Basal ROS (BROS) and stimulated ROS (SROS) production were measured by flow cytometry (Becton Dickinson, San Jose, CA) using the two-color method. ROS production was measured using the ROS-reacting fluorescent agent, hydroethidine (HE; PolyScience Inc., Warrington, PA). HE redox-sensitive probes have been widely used to detect intracellular superoxide anions.[26]

The concentration of hydroethidine was adjusted to 44.4 μM and used as an ROS marker in neutrophils (HE reagent). As a stimulant, zymosan (OZ; Sigma-Aldrich, St. Louis, MO), a yeast cell line (*Saccharomyces cerevisiae*) opsonized with healthy adult pooled serum, was adjusted to 5 mg/ml. A whole blood rising kit (Beckman Coulter Inc., Miami, FL) was used as a hemolytic agent, and the hemolytic agent stock solution from the kit was diluted 25-fold with phosphate-buffered saline (PBS) according to the manufacturer's instructions to prepare a required amount.

Two tubes for BROS and SROS were prepared for each person, and 100 μl of heparin-collected whole blood and 22 μl of HE reagent were mixed well and incubated at 37°C for 5 min. After the incubation was completed, 25 μl of the stimulating reagent OZ was added to the SROS measurement tube, mixed well, and incubated at 37°C for 35 min. After incubation, 1.0 ml of hemolytic agent was added and stirred into the solution for both SROS and BROS. Next, 250 μl of the fixative in the kit was added and stirred into the solution as soon as it became transparent, and the mixture was allowed to stand for 5 min. After the hemolysis was completed, the cells were washed twice with PBS containing sodium azide. Subsequently, 50 μl of 5% paraformaldehyde was added.

During flow cytometry, neutrophils were irradiated with a 488-nm laser beam generated from a 15-mW argon laser with forward- and side-scattering emissions, which were simultaneously recorded. Green fluorescence generated from fluorescein isothiocyanate was detected using a 530-nm filter, and orange fluorescence generated from HE was detected using a 585-nm filter. Fluorescence intensity (FI) was measured as the number of neutrophils per 10,000 screened from the forward- and side-scattering emissions for each sample. The cumulative FI, that is, the sum of the FI values multiplied by the percentage of positive cells, was used as a quantitative index.

In the present study, superoxide production was used as an index of ROS production. Superoxide is an upstream substance in ROS metabolism and all ROS are metabolites of superoxide. Accordingly, the amount of superoxide produced reflects the total production of ROS.

Statistical analysis. Categorical variables are presented as frequencies and continuous variables as medians with interquartile ranges. Comparisons between the two groups were made using χ-square and Mann–Whitney *U* tests for independence. The Pearson's correlation coefficient was used to determine the relationship between neutrophil function and liver fibrosis-related parameters. A multiple regression model with BROS, SROS, FIB-4 index, APRI, NFS, age by group, and sex was used for predictive analysis. The selection of independent variables was based on previous studies and included BMI, exercise, smoking, and drinking habits.[27–31] We controlled for these confounders to clarify the relationship between neutrophil function and liver fibrosis. Prior to the statistical analysis of liver fibrosis and neutrophil function, all parameters were log-transformed (natural logarithm) to approximate a normal distribution.

Statistical analyses of the clinical data were performed using the Statistical Package for the Social Sciences (SPSS) ver. 28.0 (IBM Corp., Armonk, NY). Statistical significance was set at $p<0.05$.

Ethics statement. This study was performed in accordance with the ethical standards of the Declaration of Helsinki and was approved by the medical ethics committee of Hirosaki University

Table 1. Clinical characteristics of the study participants

	Male			Female		
	≤64 years (*n* = 289)	≥65 years (*n* = 100)	*p* value	≤64 years (*n* = 425)	≥65 years (*n* = 186)	*p* value
Age (years)	46.0 (35.5–56.0)	68.0 (66.0–75.0)	<0.001	50.0 (38.0–58.5)	70.5 (67.0–75.0)	<0.001
BMI (kg/m^2)	23.3 (21.6–25.5)	23.7 (21.8–25.7)	0.708	21.2 (19.4–23.8)	22.7 (21.0–24.8)	<0.001
FIB4 index	0.853 (0.65–1.19)	1.78 (1.44–2.24)	<0.001	0.966 (0.69–1.32)	1.906 (1.51–2.33)	<0.001
APRI	0.241 (0.19–0.30)	0.274 (0.22–0.37)	<0.001	0.197 (0.16–0.25)	0.272 (0.21–0.34)	<0.001
NFS	–0.114 (–0.62–0.48)	1.298 (0.95–1.80)	<0.001	0.198 (–0.28–0.65)	1.34 (0.99–1.79)	<0.001
BROS (×10^3)	1.14 (0.69–1.74)	1.19 (0.85–1.80)	0.229	1.20 (0.76–1.90)	1.32 (0.81–2.13)	0.131
SROS (×10^5)	5.56 (4.65–7.10)	5.66 (4.61–7.06)	0.972	5.44 (4.46–6.54)	5.21 (4.11–6.73)	0.300
WBC (×10^3/μl)	5.4 (4.50–6.40)	5 (4.30–6.00)	0.153	4.60 (3.90–5.60)	4.60 (3.80–5.60)	0.483
Neutrophil (×10^3/μl)	2.94 (2.32–3.62)	2.99 (2.39–3.59)	0.49	2.52 (2.05–3.25)	2.58 (2.02–3.19)	0.963
Platelet (×10^4/μl)	23.4 (20.5–26.6)	20.7 (18.4–23.5)	<0.001	24.1 (20.9–28.0)	21.3 (18.5–25.8)	<0.001
Albumin (g/dl)	4.6 (4.5–4.9)	4.4 (4.2–4.5)	<0.001	4.5 (4.3–4.6)	4.4 (4.2–4.6)	0.041
AST (IU/L)	22.0 (19.0–27.0)	23.5 (19.0–29.0)	0.189	19.0 (16.0–23.0)	23.0 (20.0–26.0)	<0.001
ALT (IU/L)	23.0 (18.0–31.0)	19.0 (16.0–26.0)	0.002	15.0 (11.5–19.0)	16.0 (13.0–21.0)	<0.001
GGT (IU/L)	35.0 (22.0–59.0)	26.0 (20.3–41.5)	0.002	17.0 (14.0–24.5)	18.0 (15.0–27.0)	0.075
Total bilirubin (mg/dl)	0.8 (0.7–1.1)	0.9 (0.7–1.1)	0.846	0.8 (0.6–1.0)	0.8 (0.7–0.9)	0.923
Diabetes or Impaired glucose tolerance (*n*)	21 (7.3%)	16 (16%)	0.016	9 (2.1%)	19 (10.2%)	<0.001
Exercise habit (*n*)	36 (12.5%)	28 (28%)	<0.001	43 (10.1%)	31 (16.7%)	0.032
Smoking habit (*n*)	102 (35.3%)	16 (16%)	<0.001	54 (12.7%)	6 (3.2%)	<0.001
Drinking habit (*n*)	199 (68.9%)	67 (67%)	0.826	146 (34.4%)	29 (15.6%)	<0.001

Data are presented as numbers (%) or median (range). BMI, body mass index; NFS, non-alcoholic fatty liver disease fibrosis score; APRI, aspartate aminotransferase to platelet ratio index; BROS, basal reactive oxygen species; SROS, stimulated reactive oxygen species; WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GTT, glutamyl transpeptidase.

(authorization number: 2014-377). All the patients provided written informed consent to participate in this study.

Results

Characteristics of the study participants. In total, 389 males [median age 53.0 (37.0–65.0) years] and 611 females [median age 57.0 (42.0–66.0) years] were enrolled in the study (*n* = 1,000; range, 19–91 years.). Participants were then divided into four groups based on sex (male; female) and age (young, <65 years; old, ≥65 years) as follows: male, young (*n* = 289); male, old (*n* = 100); female, young (*n* = 425); female, old (*n* = 186).

The participants' characteristics are shown in Table 1. In both males and females, the FIB-4 index, APRI, and NFS were significantly higher in the older groups than in the younger groups. However, there were no significant differences in the neutrophil count, BROS, or SROS between the older and younger groups in either males or females.

Relationship between ROS and liver fibrosis (correlation coefficient). Table 2 and 3 summarize the results of the correlation between neutrophil function and liver fibrosis by participant age group. Single correlation analysis revealed that BROS had a positive correlation with the FIB-4 index and APRI and a negative correlation with platelets in males and females in the younger groups. A positive correlation between BROS and AST levels was also observed in females in the younger groups (Table 2). A positive correlation with SROS and GGT in males in the younger group and a negative correlation with SROS, NFS, and BMI were observed in females in the older group (Table 3).

Relationship between ROS and liver fibrosis (multiple regression analysis). Multiple regression analysis was performed, where the dependent variable was ROS, and the independent variables were BMI, exercise, smoking, and drinking habits, in addition to liver fibrosis scoring. The results are presented in Table 4 and 5. Multiple linear analyses showed a significant positive correlation between BROS and the FIB-4 index, APRI, and NFS in males and females in the younger group (Table 4). A significant negative correlation between SROS and NFS was observed in females in both the younger and older groups (Table 5). In contrast, no relationship was observed between ROS levels and liver fibrosis in males in the older group.

Discussion

In this study, we revealed that neutrophil-generated BROS increased in males and females in the younger groups, and SROS in females decreased with increasing degrees of liver fibrosis. Furthermore, multivariate analysis adjusted for body size, and lifestyle, which influence neutrophil function, showed similar results. A visual summary of the main findings of this study is shown in Fig. 2.

The FIB-4 index, APRI, and NFS, an index of liver fibrosis, all showed positive correlations with BROS, an index of basal neutrophil ROS production, in this study. Similar results were also observed in the multivariate analysis adjusted for BMI, exercise, smoking, and drinking habits that affect neutrophil function and liver fibrosis. Chronic neutrophil ROS production causes oxidative stress and contributes to the development of atherosclerosis, diabetes, lung toxicity, and malignant tumors.[16,17] Similarly, NAFLD/NASH is recognized as a phenotype of metabolic syndrome in the liver and is known to be a risk factor for atherosclerotic diseases such as cerebrocardiovascular disease along with hypertension, diabetes and dyslipidemia; the importance of liver fibrosis as a cause of these diseases has been widely established.[32–34] Hence, neutrophil-induced oxidative stress and liver fibrosis may be associated with the development of lifestyle-related diseases such as atherosclerosis, and this association has been reported in histological studies.[22] Oxidative low density lipoprotein, which is involved in atherosclerotic

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Table 2. Relationships between BROS and liver fibrosis-related parameters (correlation coefficients)

	Male				Female			
	≤64 years		≥65 years		≤64 years		≥65 years	
	r	*p* value	r	*p* value	r	*p* value	r	*p* value
Age	0.890	0.132	–0.009	0.926	0.081	0.097	–0.042	0.565
BMI	–0.099	0.093	–0.049	0.628	–0.064	0.187	–0.060	0.418
FIB4 index	0.239	<0.001	0.025	0.809	0.133	0.006	0.080	0.276
APRI	0.179	0.002	0.041	0.686	0.134	0.006	0.121	0.099
NFS	0.082	0.162	0.018	0.860	0.053	0.276	–0.019	0.793
Platelet	–0.180	0.002	0.090	0.375	–0.099	0.006	–0.073	0.324
Albumin	–0.022	0.707	0.024	0.811	–0.039	0.418	–0.142	0.053
AST	0.105	0.074	0.105	0.299	0.102	0.036	0.102	0.165
ALT	–0.049	0.404	0.037	0.718	0.063	0.195	0.070	0.341
GGT	0.006	0.924	0.133	0.186	0.047	0.338	–0.060	0.417
Total bilirubin	0.118	0.045	–0.015	0.881	–0.014	0.778	0.095	0.198

Pearson's correlation coefficients between neutrophil-generated BROS production (CFI) and liver fibrosis-related parameters. BROS, basal reactive oxygen species; BMI, body mass index; NFS, non-alcoholic fatty liver disease fibrosis score; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GTT, γ-glutamyl transpeptidase.

Table 3. Relationships between SROS and liver fibrosis-related parameters (correlation coefficients)

	Male				Female			
	≤64 years		≥65 years		≤64 years		≥65 years	
	r	*p* value	r	*p* value	r	*p* value	r	*p* value
Age	0.041	0.483	–0.093	0.357	–0.077	0.113	–0.115	0.118
BMI	–0.067	0.257	0.154	0.126	0.029	0.551	–0.151	0.039
FIB4 index	0.006	0.922	0.043	0.669	–0.039	0.427	–0.024	0.749
APRI	0.006	0.920	0.041	0.684	0.004	0.936	0.052	0.478
NFS	0.006	0.920	0.068	0.503	–0.080	0.101	–0.253	<0.001
Platelet	0.083	0.158	–0.093	0.357	–0.020	0.675	–0.005	0.950
Albumin	–0.019	0.751	0.077	0.444	0.051	0.295	0.113	0.126
AST	0.057	0.335	–0.005	0.964	–0.012	0.804	0.070	0.344
ALT	0.046	0.436	–0.026	0.797	–0.012	0.805	0.091	0.218
GGT	0.143	0.015	0.141	0.163	–0.010	0.838	0.034	0.643
Total bilirubin	0.002	0.966	0.041	0.683	0.042	0.387	0.131	0.076

Pearson's correlation coefficients between neutrophil-generated SROS production (CFI) and liver fibrosis-related parameters. SROS, stimulated reactive oxygen species; BMI, body mass index; NFS, non-alcoholic fatty liver disease fibrosis score; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GTT, γ-glutamyl transpeptidase.

plaque development and destabilization, is produced by neutrophil MPO. Ikura *et al.*,[22] reported that oxidized phosphatidylcholine, a component of oxidized low-density lipoprotein and a type of oxidized phospholipid detected in oxidatively damaged cells, is present in NASH liver tissue and is surrounded by an infiltrate of myeloperoxidase-positive neutrophils. This finding indicated that NASH and atherosclerosis are associated with neutrophil-induced oxidative stress. Further, this study revealed that liver fibrosis may have harmful effects not only on the liver but also on the whole body through the increased production of basal neutrophil-generated ROS.

The FIB-4 index, APRI, and NFS included AST and platelets in their formulas and found a positive correlation for AST and a negative correlation for platelets with BROS in a single correlation analysis. In contrast, age, BMI, ALT, GGT, and total bilirubin levels were not associated with BROS. AST in hepatocytes reflects the degree of hepatic inflammation because it is released into the blood when hepatocytes are damaged. On the other hand, platelets have been reported to inhibit liver fibrosis via activation of the hepatocyte growth factor (HGF)/Met pathway, with thrombocytopenia promoting liver fibrosis.[35] Elevated AST levels and decreased platelet counts may be major factors in the relationship between liver fibrosis and BROS.

In this study, no association was found between liver fibrosis and BROS in either males or females in the older age groups. ROS exert various detrimental effects via inflammatory cytokines such as tumor necrosis factor-α, which increase with age.[36] Accordingly, no association between liver fibrosis and BROS was observed in the older groups due to the influence of higher levels of inflammatory cytokines in this group than those seen in the younger groups.

In this study, a negative correlation was observed between the NFS and SROS in females. Because SROS reflect the ability to eliminate foreign substances, a decrease in SROS suggests a reduced immune capacity against infection.[16] Liver fibrosis not only decreases the number of neutrophils, but also reduces phagocytosis and bactericidal activity against pathogens, resulting in increased susceptibility to infections.[37,38] Decreased neutrophil SROS production may be related to a lack of resistance to infection through liver fibrosis.

Table 4. Multiple linear regression analysis with BROS as the dependent variable

	Male						Female					
	≤64 years			≥65 years			≤64 years			≥65 years		
	β	*p* value	R^2	β	*p* value	R^2	β	*p* value	R^2	β	*p* value	R^2
FIB-4	0.231	<0.001	0.076	−0.027	0.805	0.023	0.118	0.017	0.029	0.056	0.462	0.023
APRI	0.188	0.002	0.061	−0.003	0.976	0.023	0.118	0.016	0.029	0.110	0.142	0.032
NFS	0.126	0.045	0.041	0.007	0.947	0.023	0.113	0.048	0.025	−0.024	0.768	0.020

Multiple regression analysis of the neutrophil-generated BROS (CFI) and liver fibrosis scores. β, standardized coefficient; R^2, coefficient of determination; NFS, non-alcoholic fatty liver disease fibrosis score; APRI, aspartate aminotransferase to platelet ratio index. Forced-entry multiple regression was performed by sex using BROS production as the dependent variable and BMI, exercise habits, smoking, and drinking as independent variables.

Table 5. Multiple linear regression analysis with SROS as the dependent variable

	Male						Female					
	≤64 years			≥65 years			≤64 years			≥65 years		
	β	*p* value	R^2	β	*p* value	R^2	β	*p* value	R^2	β	*p* value	R^2
FIB-4	−0.016	0.791	0.029	0.021	0.845	0.058	−0.040	0.470	0.006	−0.024	0.748	0.038
APRI	−0.002	0.972	0.028	−0.005	0.959	0.058	0.008	0.879	0.004	0.069	0.351	0.042
NFS	0.008	0.899	0.028	0.017	0.877	0.058	−0.130	0.023	0.017	−0.212	0.007	0.076

Multiple regression analysis of the neutrophil-generated SROS (CFI) and liver fibrosis scores. β, standardized coefficient; R^2, coefficient of determination; NFS, non-alcoholic fatty liver disease fibrosis score; APRI, aspartate aminotransferase to platelet ratio index. Forced-entry multiple regression was performed by sex using SROS production as the dependent variable and BMI, exercise habits, smoking, and drinking as independent variables.

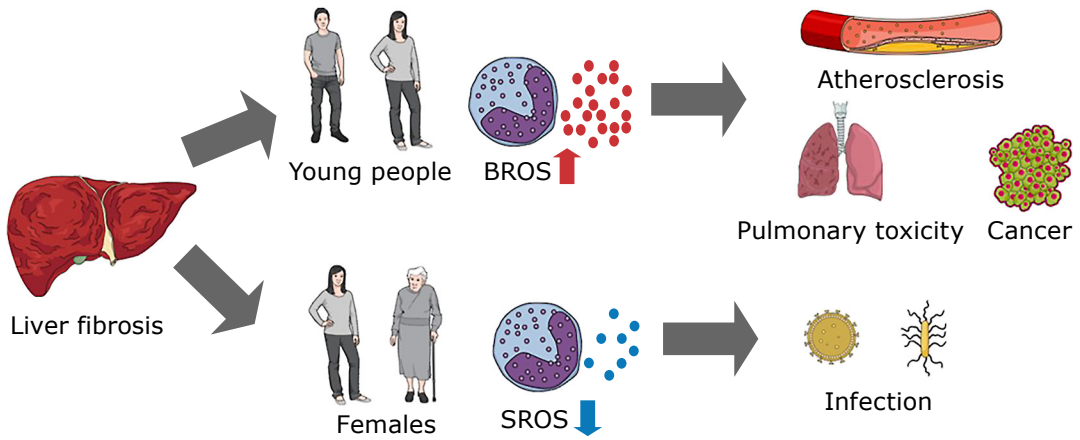

Fig. 2. A visual summary of the main findings of this study. Liver fibrosis increases neutrophil-generated BROS levels in both males and females in younger people and may be associated with the development of lifestyle-related diseases such as atherosclerosis, pulmonary toxicity, and cancer. On the other hand, liver fibrosis decreases neutrophil-generated SROS levels in females and may be related to a lack of resistance to infection. BROS, basal reactive oxygen species; SROS, stimulated reactive oxygen species.

Moreover, no association was found between APRI, FIB-4 index, and SROS. The NFS formula includes BMI, glucose tolerance, and albumin levels, which are not included in the APRI or FIB-4 index. This study revealed a single negative correlation between BMI and SROS levels in females in the older groups. Obese individuals are more susceptible to infection because of a decrease in adiponectin, which prevents an increase in neutrophils during infection via increased tumor necrosis factor-α.[39] The association between liver fibrosis and SROS may be strongly influenced by an increase in inflammatory cytokines due to a high BMI.

Unlike in females, no association between liver fibrosis and SROS was found in males, indicating that sex hormones might be a factor. Sex hormones influence immune function, especially female hormones, and are involved in various aspects of the immune system, including oxidative stress.[40,41] Previous studies have reported that females tend to have higher immune functions and higher neutrophil functions than males.[42–44] This current study revealed that liver fibrosis leads to increased susceptibility to infections, which may be more pronounced in females.

This study has several limitations. First, our study population was geographically limited to a district in Japan; therefore, it cannot be generalized to all ethnicities. Second, the diagnosis of liver fibrosis was reached using scoring that was calculated with items measured during a health checkup and not through a liver biopsy, which is an invasive procedure. In a study of the general population, it was unfeasible to perform liver biopsies.

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Conclusion

Liver fibrosis increases neutrophil-generated BROS levels in both males and females in younger groups. In addition, liver fibrosis decreases neutrophil SROS levels in females. Preventing liver fibrosis may prevent lifestyle-related diseases and infections through neutrophil-related immune system enhancement.

Acknowledgments

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Abbreviations

ALT	alanine aminotransferase
APRI	aspartate aminotransferase to platelet ratio index
AST	aspartate aminotransferase
BMI	body mass index
BROS	basal reactive oxygen species
FI	fluorescence intensity
FIB-4	fibrosis 4
GTT	glutamyl transpeptidase
HbA1C	hemoglobin A1c
HE	hydroethidine
MPO	myeloperoxidase
NADPH	nicotinamide adenine dinucleotide phosphate
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NFS	non-alcoholic fatty liver disease fibrosis score
ROS	reactive oxygen species
SROS	stimulated reactive oxygen species
WBC	white blood cell

Conflict of Interest

No potential conflicts of interest were disclosed.

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