Received: 13 March 2023 | Accepted: 8 May 2023

DOI: 10.1111/acer.15103

ALCOHOL CLINICAL & EXPERIMENTAL RESEARCH ISBRA

RESEARCH ARTICLE

Inflammatory cytokines in alcohol use disorder patients are lower in smokers and users of smokeless tobacco

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Funding information
The Research Council of Norway, Grant/Award Number: 251140

Abstract

Background: Smoking and alcohol use often co-occur, and the use of nicotine-containing products is particularly common among persons with alcohol use disorder (AUD). Recent evidence shows that chronic alcohol use leads to inflammation through increased gut permeability and dysregulated cytokine levels. While cigarette smoking also has detrimental health effects, nicotine has immune dampening effects in some settings. Preclinical evidence demonstrates that nicotine can dampen alcohol-induced inflammation, but inflammatory responses after nicotine use has not been studied in persons with AUD. This study compared the level of circulating cytokines in abstinent AUD inpatients who were non-tobacco users, smokers, users of Swedish snus, or dual tobacco users.

Methods: We collected blood samples and information about somatic and mental health and tobacco habits from 111 patients in residential treatment for AUD and 69 healthy controls. Levels of interferon (IFN)-γ, interleukin (IL)-10, tumor necrosis factor (TNF)-α, IL-17a, IL-1β, IL-6, IL-8, IL-1 receptor antagonist (ra), and monocyte chemoattractant protein (MCP)-1 were examined using a multiplex assay.

Results: Patients with AUD had higher levels of seven cytokines than healthy controls. Among the AUD patients, nicotine users had lower levels of IL-10, TNF-α, IL-17a, IL-1β, IL-8, and MCP-1 (all $p < 0.05$).

Conclusions: Our findings may indicate that nicotine has anti-inflammatory effects in patients with AUD. Nonetheless, nicotine use cannot be recommended as a viable therapeutic option to reduce alcohol-induced inflammation because of its other adverse effects. Additional studies of the effects of tobacco or nicotine products on cytokine patterns in relation to mental or somatic health conditions are warranted.

KEYWORDS
alcohol, alcohol use disorder, cytokines, inflammation, nicotine

INTRODUCTION

The use of alcohol and tobacco often go together, both for occasional and chronic use. Heavy use of alcohol tends to coincide with heavy cigarette smoking (Shiffman & Balabanis, 1996). There is a range of theories for this concurrent use, including shared genetics leading to addictive behavior, epigenetic factors, pharmacological interactions, and the drugs counteracting each other's adverse effects (Hurley et al., 2012).

While moderate ethanol (EtOH) consumption may dampen the immune system (Barr et al., 2016; Romeo et al., 2007), chronic and excessive use may activate it and increase inflammatory responses (Adams et al., 2020; Barr et al., 2016). Alcohol use disorder (AUD) might therefore also be viewed as an inflammatory disorder (González-Reimers et al., 2014). Recent evidence shows that EtOH exposure increases gut permeability, enabling gut-derived bacterial components (e.g., lipopolysaccharides and peptidoglycan), to cross the intestinal barrier, enter the systemic circulation, affect organs (e.g., the liver and the brain), and lead to neuroinflammation through increased levels of pro-inflammatory cytokines (Calleja-Conde et al., 2021; Leclercq et al., 2017). Further, the resulting dysregulation of cytokines is associated with mental health problems, such as depression (Maes, 1995; Troubat et al., 2021). Although this dysregulation of cytokine levels is most pronounced during active drinking, studies show that chronic heavy drinking may have long-term impact on the immune system (Pasala et al., 2015), and cytokine levels among AUD patients and control subjects are still significantly different even after weeks of abstinence (Adams et al., 2020). Interestingly, several studies have found that these immune system alterations, particularly in the central nervous system, in turn may influence drinking behavior and lead to increased consumption and progress the stages of addiction (Blednov et al., 2011; Crews et al., 2017).

Cigarette smoking impacts both innate and adaptive immunity and may play a dual role in regulating immunity by either exacerbation of pathogenic immune responses or attenuation of defensive immunity (Qiu et al., 2017). While smoking represents a major health risk, nicotine as such may have a less profound immunomodulating effect (Tran et al., 2021). Nicotine has been shown to ameliorate inflammation in ulcerative colitis while results are still inconclusive for other autoimmune conditions (e.g. lupus erythematosus, rheumatoid arthritis, and multiple sclerosis; Piao et al., 2009; Zhang et al., 2022). One mechanism of action is that nicotine acts on the α7 nicotinic acetylcholine receptor (α7-nAChR) which is presented on immune cells like macrophages and T cells and has a broad influence on immune response (Cox et al., 2020; Nizri et al., 2009).

Swedish snus (a smokeless tobacco product containing fine-ground tobacco leaves) is generally considered a safer nicotine use option than cigarette smoking as studies show less increased risk of overall mortality or morbidity from cardiovascular disease or cancers compared to smoking (Hajat et al., 2021; Rostron et al., 2018). Snus is used by 15% of Norwegians aged 16 to 24 years, which is about twice the number of cigarette smokers (Statistics Norway, 2022). However, most studies of immune system effects of nicotine in humans so far has looked at the effects of cigarette smoking (Qiu et al., 2017), and only some include use of smokeless tobacco (Tran et al., 2021). As the combustion process of smoking is thought to cause many of the detrimental effects of smoking (Soleimani et al., 2022), it is of interest to also investigate the effects of noncombustible nicotine products and explore differences between the two.

In a preclinical study, Kalejaiye et al. (2017) found that EtOH-induced increase in pro-inflammatory cytokines (interleukin 1 beta [IL-1β] and tumor necrosis factor alpha [TNF-α]) in the hippocampus was blocked by nicotine coadministration—(Kalejaiye et al., 2013). Furthermore, they observed that depressive-like behaviors induced by EtOH were normalized. This is of interest due to the possible link between immune activation and major depression (Hurley & Tizabi, 2013). Although several studies have examined co-use of cigarettes and alcohol and described interactions, the combined effect of tobacco and alcohol use on immune responses in humans is scarcely studied (Hurley et al., 2012).

The main aim of this study was to compare the levels of circulating cytokines in nontobacco users, smokers, users of Swedish snus, and dual tobacco users among abstinent AUD inpatients.

MATERIALS AND METHODS

Study participants

Patients were recruited from three different inpatient rehabilitation clinics in Eastern Norway. The material has previously been described elsewhere (Bolstad et al., 2021). Patients with AUD diagnosis according to the International Classification of Diseases 10th Revision (ICD-10) were considered for participation. Patients with psychosis, cognitive impairment, severe somatic illness, or unfamiliarity with a Scandinavian language were not eligible for participation. The eligible patients were provided with information about the study and 111 patients signed written informed consent. Cross-sectional data were collected at approximately 1 week after entry into the clinics. Information about mental health, substance dependence, and tobacco use was obtained during an interview conducted by trained staff. Information about mental distress, physical activity, sleep quality, and alcohol dependence were collected using self-report forms.

A group of healthy controls were recruited from a blood donation center. Healthy individuals accepted as blood donors were included in the study ($n = 69$).

Measures

Information about age, sex, and educational level was collected initially when meeting the participant.

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Blood collection and cytokine measurements

Venous blood samples were collected using 6 mL ethylenediaminetetraacetic acid vacutainer blood collection tubes. The tubes were then turned upside down 10 times and centrifuged at 1500 × g. The plasma was then transferred with a pipette into Eppendorf microtubes and stored for a short time on site at −20°C before storage at −80°C until use.

The panel of cytokines was chosen based upon previous literature about inflammatory effects of chronic alcohol use. After thawing, 25 μL human plasma was diluted (1:5) and then analyzed. Cytokines were measured on a MESO® QuickPlex SQ 120 Multiplex Imager using the custom U-PLEX Biomarker Group 1 Multiplex assay from MSD, Rockville, Maryland, USA. A 9-plex assay returned data on interferon (IFN)-γ, interleukin (IL)-10, tumor necrosis factor (TNF)-α, IL-17a, IL-1β, IL-6, IL-8, IL-1 receptor antagonist (ra), and monocyte chemoattractant protein (MCP)-1. Analyses were performed at Vitas Analytical Laboratory AS, Oslo, Norway.

Tobacco habits

Information about tobacco use habits was collected by interview questions. The participants were asked "Do you smoke cigarettes?" and "Do you use snus?", with follow-up questions "How often?" if they confirmed use of cigarettes or snus. Smokers/users of snus hereafter refers to those who reported daily use, whereas those who reported occasional use are referred to as nonsmokers or nonusers of snus. The participants were also asked whether they had previously smoked cigarettes daily, and if so, time elapsed since cessation.

Mental health and substance use measures

Mental distress was measured by the Hopkins Symptom Checklist 10 question version (HSCL-10; Derogatis et al., 1974). The HSCL-10 is a widely used self-report tool that covers common symptoms of depression and anxiety experienced the past week. There are 10 four-level Likert items (Not at all (1), A little (2), Quite a bit (3), and Extremely (4)) which are summed up and divided by number of endorsed items yielding an individual score between 1 and 4 where higher score corresponds to higher level of mental distress.

The Mini International Neuropsychiatric Interview (M.I.N.I.) version 6.0 was used to identify substance use disorder.

AUD severity was measured using the Norwegian version of the Severity of Dependence Scale (SDS; Gossop et al., 1995; Kristoffersen et al., 2019). The SDS consists of five 4-level (0 to 3) Likert items mapping impaired control over drinking, anxiety, and preoccupation with drinking the past year. Item responses are summed up into a score where higher score indicates more severe AUD.

Somatic health and medication

All participants were asked about chronic illness and acute illness past 2 weeks at baseline and follow-up meetings. Lists of inflammatory illnesses and anti-inflammatory medication that were included as variables are presented in Tables S1 and S2.

Lifestyle measures

International Physical Activity Questionnaire short version (IPAQ-S) was used to measure the level of physical activity (Craig et al., 2003; Kurtze et al., 2008). Time spent walking or doing moderate or vigorous exercise weekly was reported in a 7-item questionnaire, and the participants were placed in categories of low, moderate, or high physical activity level. The variable was dichotomized as low versus moderate/high for the purpose of this study.

Quality of sleep was measured using the Sleep Condition Indicator (Espie et al., 2014).

Statistical analyses

Sociodemographic data at baseline were assessed using descriptive statistics and group differences were tested using Pearson's chi-square test and Wilcoxon rank-sum test for categorical and continuous variables, respectively. Spearman's correlation and Wilcoxon rank-sum test were used to measure the associations between background variables and the nine cytokines. Linear regression was used to assess associations between tobacco use as predictor (smoke, snus, or dual compared to no tobacco use) and the nine cytokines as outcomes. These variables were first entered into unadjusted analyses, before adding age and sex (Model 1), somatic inflammatory illness, body mass index (BMI), and level of physical activity (Model 2), and SDS and HSCL-10 (Model 3) as adjustment variables. Adjustment variables were included in the regression analyses if the association in the bivariate analysis was significant with a *p*-value below 0.05. Analyses were performed using Stata version 17.

RESULTS

Table 1 shows description of background variables for patient and control groups stratified by types of nicotine use. The patients who both smoked and used snus (dual) were less often male ($p < 0.05$) and were younger ($p < 0.05$) than the non-tobacco group. Patients that either smoked ($p < 0.01$) or both smoked and used snus ($p < 0.05$) had lower university level education than non-tobacco users. Snus-using patients had a lower alcohol SDS than non-tobacco users ($p < 0.05$). There were no statistically significant differences between non-tobacco-users and snus-users in the control group.

There were higher levels of IFN-γ, IL-1β, IL-6, IL-8, and MCP-1 ($p < 0.001$), and IL-17 and IL-1ra ($p < 0.05$) among patients than

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TABLE 1 Background variables for the AUD patients ($N = 111$) with four different tobacco consumption habits and for the two groups of controls ($N = 69$).

		Patient group					Control group		
		All	No tobacco	Smoke	Snus	Dual	All	No tobacco	Snus
		$n = 111$	$n = 11$	$n = 69$	$n = 15$	$n = 16$	$n = 69$	$n = 59$	$n = 10$
Sociodemographics									
Sex (female)	*n* (%)	30 (27)	5 (45)	20 (29)	4 (27)	1 (6)†	25 (36)	19 (32)	6 (60)
Age (years)	Median (IQR)	53 (44–58)*	57 (47–61)	54 (50–58)	45 (34–56)	39 (32–54)†	49 (37–57)	46 (36–57)	52 (49–58)
Education (university degree)	*n* (%)	24 (33)**	8 (80)	6 (12)††	7 (58)	3 (25)†	36 (27)	31 (53)	5 (50)
Smoking history									
Smoked before	*n* (%)	–	4 (36)	–	5 (33)	–	–	20 (34)	1 (10)
Time since smoking cessation (years)	Median (IQR)	–	9.5 (3.5–16.0)	–	9.0 (3.0–15.0)	–	–	12.0 (6.5–17.5)	5.0 (–)
Somatic health									
BMI (kg/m^2)	Median (IQR)	26.5 (23.5–29.3)	28.5 (25.6–31.8)	26.1 (23.1–28.9)	27.0 (22.4–29.0)	27.0 (25.1–29.1)	26.3 (24.8–29.1)	26.5 (24.7–29.4)	25.4 (25.1–27.5)
Waist measure (cm)	Median (IQR)	101 (90–110)***	105 (92–115)	101 (90–110)	100 (85–110)	101 (94–111)	92 (85–103)	92 (85–104)	90 (81–98)
Obese	*n* (%)	23 (21)	5 (45)	14 (20)	2 (13)	2 (13)	14 (20)	13 (22)	1 (10)
No chronic or acute infl. illness	*n* (%)	51 (46)	5 (45)	32 (46)	6 (40)	8 (50)	–	–	–
Anti-inflammatory medication use	*n* (%)	65 (59)	7 (64)	40 (58)	8 (53)	10 (63)	–	–	–
CRP ≥5	Median (IQR)	27 (25)***	6 (55)	11 (16)††	2 (13)†	8 (50)	2 (3)	2 (3)	0 (0)
Lifestyle									
Low level of physical activity	*n* (%)	43 (55)**	4 (44)	29 (62)	3 (30)	7 (58)	13 (21)	12 (23)	1 (11)
Quality of sleep score	Median (IQR)	16 (11–25)***	13 (10–28)	16 (11–25)	20 (14–25)	14 (10–18)	29 (25–31)	29 (27–31)	25 (23–31)
Mental health									
HSCL-10 score	Median (IQR)	2.0 (1.5–2.5)***	1.8 (1.4–2.2)	2.0 (1.5–2.6)	1.7 (1.2–2.3)	2.4 (2.0–2.6)	1.1 (1–1.3)	1.1 (1.0–1.3)	1.1 (1.1–1.3)
Substance use-related measures									
Other substance use disorder	*n* (%)	22 (20)	1 (9)	10 (14)	4 (27)	7 (44)	–	–	–
AUDIT score	Median (IQR)	29 (24–34)***	33 (28–34)	29 (22–34)	27 (25–30)	33 (29–34)	3 (2–5)	3 (2–5)	2 (2–4)
Alcohol SDS score	Median (IQR)	10 (8–12)	11 (10–11)	10 (8–12)	9 (7–10)†	11 (8–12)	–	–	–
PEth (μmol/L)	Median (IQR)	0.30 (0.11–0.60)***	0.26 (0.19–0.71)	0.30 (0.09–0.56)	0.17 (0.06–0.33)	0.61 (034–0.97)	0.00 (0.00–0.10)	0.00 (0.0–0.09)	0.02 (0.00–0.10)

TABLE 1 (Continued)

		Patient group					Control group		
		All	No tobacco	Smoke	Snus	Dual	All	No tobacco	Snus
		$n = 111$	$n = 11$	$n = 69$	$n = 15$	$n = 16$	$n = 69$	$n = 59$	$n = 10$
Time since last drink (days)	Median (IQR)	18 (13–30)***	14 (12–30)	20 (13–31)	21 (14–55)	13 (10–18)	3 (2–9)	3 (2–10)	4 (2–7)
Duration of drinking (years)	Median (IQR)	15 (7–22)	15 (7–20)	15 (9–23)	11 (5–24)	14 (8–21)	-	-	-

Note: Bivariate tests for patient group versus control group (*), and for non-tobacco use versus each tobacco use group among patients and among controls (†). Reported as count (%) for categorical variables and median and 25th and 75th percentiles (interquartile range [IQR]) for continuous variables. $*/^{\dagger}p < 0.05$; $**/^{\dagger\dagger}p < 0.01$, and $***/^{\dagger\dagger\dagger}p < 0.001$.

healthy controls, while there was no statistically significant difference between groups for IL-10 and TNF-α levels (Table 2).

Associations between nine cytokines/chemokine and background variables among the patients are shown in Table 3. Males had significantly higher levels of IL-8 ($p < 0.05$) and MCP-1 ($p < 0.01$) than females. Age was positively related to levels of TNF-α ($p < 0.01$), IL-17 ($p < 0.05$), IL-6 ($p < 0.01$), and IL-8 ($p < 0.01$). There were moderate positive correlations between BMI, waist measure, and obesity and IL-6 and IL-1ra levels (all $p < 0.001$). Obesity was also related to higher levels of IL-10 ($p < 0.01$) and TNF-α ($p < 0.05$). Patients with an acute or chronic inflammatory illness had higher levels of IL-10 and IL-17a. Patients with low levels of physical activity had lower level of IL-1β and higher levels of IL-6, than patients who engaged in moderate or high levels of physical activity. Levels of C-reactive protein (CRP) correlated with levels of IL-10, TNF-α, IL-6, and IL-1ra. There were weak correlations between level of IFN-γ with PEth and with time since last drink, indicating that IFN-γ level decreased with the time of abstinence. None of the lifestyle or mental health-related measures were associated with any of the circulating cytokines levels.

Figure 1 shows median levels and interquartile ranges of cytokines across nicotine use groups and significant differences between non-nicotine users and smokers, snus users, or dual users, respectively, are marked. Non-nicotine users compared to different subgroups of nicotine users have higher levels for most cytokines. Levels among nicotine users are comparable to those of the control group for most cytokines.

The findings in the bivariate analysis depicted in Figure 1 were further analyzed with linear regression models for each of the cytokines (Table 4). In unadjusted analyses, lower levels were found for all cytokines except IFN-γ when comparing those using snus with those not using tobacco. Smokers also had lower levels of cytokines across the line, except for IL-6, IL-1ra, and MCP-1. After adjustment for age, sex, inflammatory illness, BMI, low physical activity, severity of dependence, and HSCL (model 3), all groups of nicotine users had lower levels of TNF-α, IL-17a, IL-1β, and IL-8 (almost significant for smokers: $p = 0.051$). In addition, snus-users had lower levels of IL-10 and MCP-1 and smokers had lower levels of IFN-γ and IL-10.

DISCUSSION

In this study, we found higher levels of seven cytokines among AUD inpatients than healthy controls. Further, among patients, levels of cytokines were lower in nicotine users (those who smoked or used snus) than nonusers. To the best of our knowledge, this is the first study to investigate the effects of nicotine on an elevated level of inflammatory markers in heavy alcohol users.

We know from several investigations that heavy alcohol use may increase the level of circulating cytokines (Adams et al., 2020). In line with this, we report increased levels of IFN-γ, IL-1β, IL-6, IL-8, MCP-1, IL-17, and IL-1ra among patients compared to healthy controls. Among the AUD patients, the nonusers of tobacco products had higher levels of several cytokines. In the adjusted linear regression,

TABLE 2 Levels of circulating cytokines in controls and patients.

	Control group	Patient group	*p*
IFN-γ	7.41 (5.28 to 9.59)	9.61 (6.73 to 12.68)	**<0.001**
IL-10	0.22 (0.18 to 0.33)	0.23 (0.18 to 0.36)	0.542
TNF-α	1.25 (1.06 to 1.59)	1.35 (1.11 to 1.75)	0.143
IL-17a	1.10 (0.72 to 1.73)	1.52 (0.80 to 2.43)	**0.030**
IL-1β	0.01 (0.01 to 0.01)	0.06 (0.01 to 0.06)	**<0.001**
IL-6	0.53 (0.40 to 0.73)	0.91 (0.66 to 1.42)	**<0.001**
IL-8	2.12 (1.73 to 2.49)	2.70 (1.82 to 3.69)	**<0.001**
IL-1ra	120.07 (89.96 to 166.09)	142.75 (100.63 to 242.44)	**0.047**
MCP-1	104.48 (91.44 to 120.98)	132.09 (102.71 to 160.16)	**<0.001**

Note: Descriptive statistics of levels of cytokines (pg/mL) are given as median and 25th and 75th percentiles. *p*-values <0.05 are shown in bold.

the circulating levels of TNF-α, IL-17a, IL-1β, and IL-8 were lower among all nicotine users, with levels close to the those observed among the healthy controls. Whereas IFN-γ, TNF-α, IL-17a, IL-1β, IL-6, and IL-8 are considered as primarily pro-inflammatory, IL-10, and IL-1ra are considered to exert anti-inflammatory action, while MCP-1 has a dual role.

We observed several associations between cytokines and background and somatic health status variables. The positive correlation observed between increasing age and TNF-α and IL-6 among patients are in line with previous reports indicating that levels of these cytokines elevate with increasing age and frailty (Michaud et al., 2013). Sex differences were also expected in regard to cytokine levels and were observed as higher levels of IL-8 and MCP-1 in males (Pennell et al., 2012). In line with previous research, we observed associations between weight-related measures and increased levels of IL-6, IL-1ra, and IL-10. IL-6 is a pro-inflammatory cytokine and is found to be increased in persons with obesity as part of the low-grade inflammation that characterizes this condition (Lakhan & Kirchgessner, 2011). Obesity is also associated with increased levels of IL-1ra—however, this cytokine is considered anti-inflammatory as it inhibits IL-1 ligands by binding nonproductively to the IL-1 receptor (Herder et al., 2009). Further, in our study, IL-10 and IL-17a were related to inflammatory illness, while IL-10, IL-6, and IL-8 increased with increasing CRP. Similarities in findings for cigarettes and snus in our study indicate that nicotine as such may be responsible for the lower cytokine levels among tobacco users. Nicotine interacts with the immune system through the α7-nAChR, which is present on immune cells, such as macrophages and dendritic cells, and is also abundant on T-cell surfaces. Nicotine has been shown to reduce T-cell proliferation and downregulate TNF-α, IFN-γ, and IL-17a in a laboratory setting (Nizri et al., 2009). TNF-α is mainly released from macrophages and levels may be expected to be lowered due to the anti-inflammatory effects of nicotine (Demirjian et al., 2006). In contrast to our results, the general finding for IL-8 is that levels are increased in smokers (Oltmanns et al., 2005). For Il-1β, findings are inconclusive as other have found the same as the current report, but also identified that circulating levels is *increased* in persons who smoke compared to those who do not (Ryder et al., 2002).

It is important to note that tobacco contains a range of other chemicals than nicotine that may influence inflammatory responses. In this study, association between alcohol use and tobacco use were observed. However, since snus and cigarettes are consumed differently and could be delivering different chemicals, nicotine may be considered as the primary psychoactive compound shared in both delivery systems.

In this study, we found that smoking, and to a lesser degree snus, reduced the levels of IFN-γ. Smoking has been shown to downregulate circulating IFN-γ levels in some patients (Raymond et al., 2021). We have found no clinical studies on the impact of smokeless tobacco on circulating INF-γ, but our results echo preclinical findings showing less impact of smokeless tobacco than of cigarettes (Liu et al., 2019; Nizri et al., 2009).

MCP-1 is a chemokine and is released by many cell types including macrophages and it is thus influenced by other cytokines. MCP-1 recruits monocytes and other immune cells to the site of inflammation during an immune response (Deshmane et al., 2009). Previous reports have shown increased level of MCP-1 in AUD (Kazmi et al., 2022), and decreased levels in healthy females who smoke (Daloee et al., 2017). Among AUD patients in our sample, the findings were mixed, showing lower MCP-1 levels among snus-users, but not in smokers or dual users. Further, both smokers and snus-users had lower levels of IL-10 in our sample. IL-10 is considered an anti-inflammatory cytokine with pleiotropic action, with one of the primary functions being inhibiting production of the pro-inflammatory cytokines TNF-α, IFN-γ, and IL-1b (Fiorentino et al., 1991).

There were no differences between nicotine users and nonusers in levels of IL-6 or IL-1ra after adjustment for background and somatic health variables. However, IL-6, IL-1ra, and in a weaker manner, IL-10, were positively correlated with BMI, and BMI seemed to account for some of the variability when entered in the regressions.

We did not detect differences in the levels of pro-inflammatory cytokines between snus users and nonusers among the healthy

TABLE 3 Associations between background variables with immune markers among the AUD patients ($N = 111$).

		Circulating cytokine								
		IFN-γ	IL-10	TNF-α	IL-17a	IL-1β	IL-6	IL-8	IL-1ra	MCP-1
Sociodemographics										
Sex (female)	z	−0.206	−0.637	1.501	−1.125	0.438	0.956	2.138*	−0.272	2.908**
Age	ρ	0.045	0.026	0.259**	0.206*	0.073	0.278**	0.260**	0.017	0.139
Somatic health										
BMI	ρ	0.086	0.230*	0.144	−0.041	−0.036	0.393***	0.010	0.435***	−0.043
Waist measure	ρ	0.077	0.139	0.172	−0.026	−0.026	0.458***	0.091	0.442***	0.063
Obesity	z	−1.244	−2.616**	−1.961*	−1.120	−1.536	−3.671***	−1.564	−3.230***	−0.531
Chronic or acute infl. illness	z	0.059	−2.778**	−1.175	−2.756**	−1.006	−1.541	0.012	0.320	−0.053
Using anti-inflammatory medication	z	1.910	−1.242	−0.802	−1.048	1.546	−0.054	−0.988	0.629	−0.377
CRP (mg/L)	ρ	0.072	0.237*	0.243*	−0.082	0.033	0.459***	0.154	0.474***	0.116
Lifestyle										
Low level of physical activity	z	0.246	−0.663	−0.633	0.707	2.088*	−2.296*	−1.160	−1.201	−0.718
Quality of sleep	ρ	0.177	−0.034	0.048	−0.014	0.152	−0.061	−0.005	−0.009	0.075
Mental health										
HSCL-10	ρ	−0.097	0.037	−0.096	−0.054	−0.175	0.015	−0.073	0.069	−0.022
Substance use-related measures										
Other substance use disorder	z	−0.118	−1.446	−0.607	−0.755	0.489	1.417	1.235	0.562	0.096
Alcohol SDS score	ρ	0.195	0.042	0.076	−0.078	−0.060	0.176	0.003	0.201	0.014
PEth (μmol/L)	ρ	0.199*	0.034	0.074	−0.024	0.174	0.119	0.073	0.144	0.056
Time since last drink: days	ρ	−0.233*	−0.046	0.004	0.068	−0.053	−0.118	−0.023	−0.162	0.011
Duration of drinking: years	ρ	0.036	−0.046	0.052	−0.014	0.053	0.101	0.141	−0.021	0.095

Note: Spearman's correlation (ρ) was used for continuous variables and Wilcoxon rank-sum test (z) was used for categorical variables. Significance level * < 0.05; ** < 0.01; and *** < 0.001.

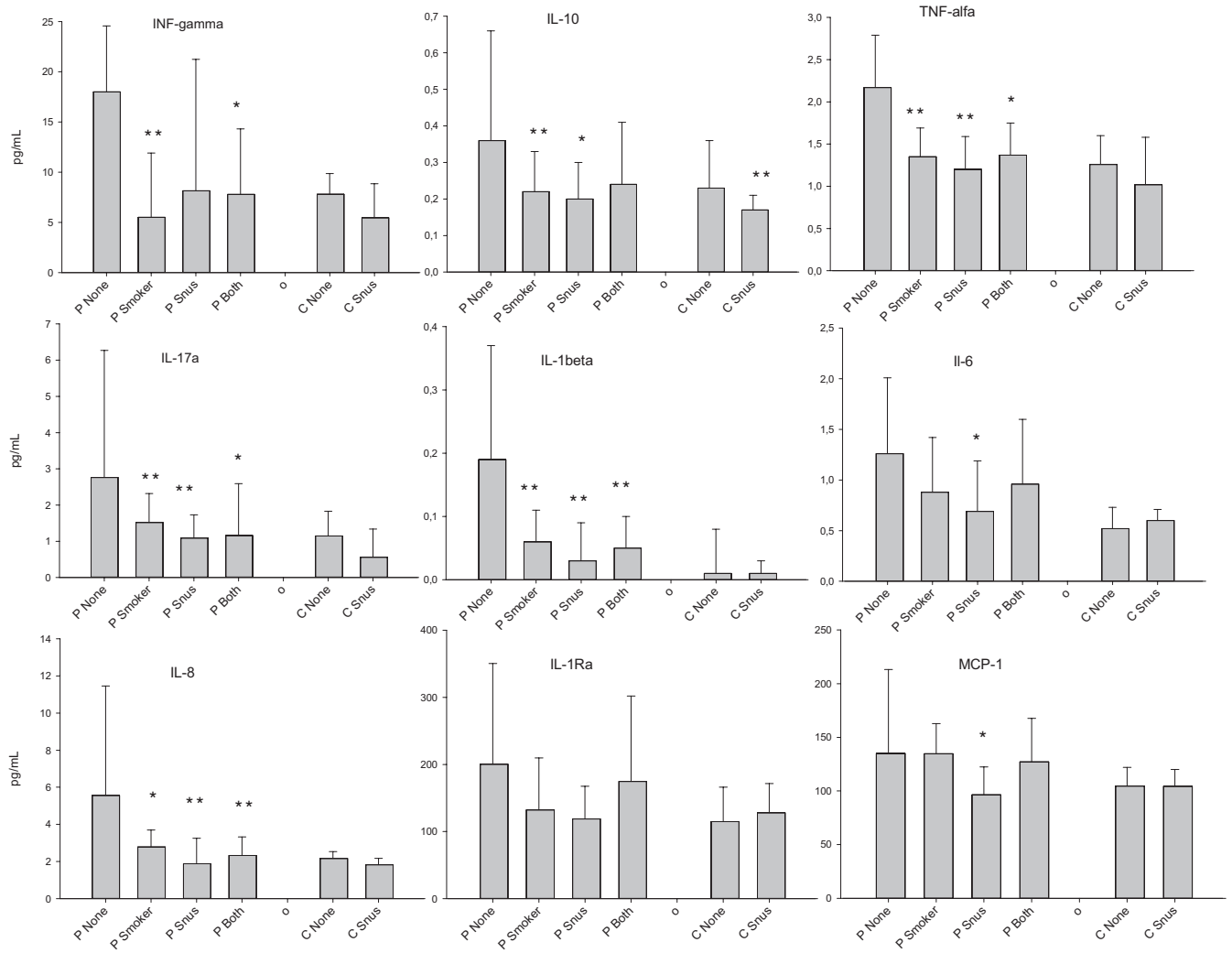

FIGURE 1 Levels of cytokines across nicotine use groups. Descriptive statistics presented in the graphs are medians and 75th percentiles. Comparisons within the patient group were performed between the non-use group and each of the tobacco groups. Comparisons within the control group were performed between non-use group and the snus-group. Significant differences ($p < 0.05$) are marked with a star.

controls, except in IL-10. Previous studies have shown comparable results (Daloee et al., 2017). This indicates that in healthy immune functioning, nicotine does not profoundly influence the immune system, whereas nicotine may dampen the immune activation seen in heavy alcohol users.

Several lines of evidence have shown strong associations between inflammation and depression (Troubat et al., 2021), and it has been suggested that both alcohol (Hurley & Tizabi, 2013) and cigarette smoking (Berk et al., 2013) are related to major depression through their influence on the immune system. A study of depression in a mouse model showed that the depressogenic effect of alcohol could be blocked by administration of nicotine (Kalejaiye et al., 2013), maybe related to changes in cytokine levels resulting from nicotine (Kalejaiye et al., 2017). This may be interesting in this context as depression rates are very high among persons with AUD, and our findings point to lower levels of inflammatory markers among nicotine users. However, we did not find statistically significant associations between mental distress and inflammatory markers in this study.

Limitations of this study includes that information on tobacco habits was limited to daily use (yes/no), as daily consumption volume was not reported. Thus, the exposure to nicotine may vary across patients, and lack of such data also prevents an exploration of a possible dose-response relationship. Information on somatic health was self-reported and relatively crude—accessing medical records would have allowed for a more accurate adjustment of the regression models. When comparing the aforementioned preclinical findings with the present results, it is important to note that the AUD patients were currently abstinent from alcohol, whereas in the preclinical models, alcohol and nicotine were coadministered. However, studies show that the impact of chronic drinking on the immune system lasts for several weeks (Adams et al., 2020). Further, none of the cytokines except IFN-γ seemed to decrease with the time of abstinence. One possible explanation for the higher level of proinflammatory cytokines among non-tobacco users could be that this group abstained from tobacco products because of somatic health issues. However, we did not find that non-tobacco users differed from the

TABLE 4 Linear regression models of association between tobacco habits and cytokine levels among the AUD patients ($N = 111$).

	Unadjusted model			Model 1			Model 2			Model 3		
	β	95% CI	*p*	*β*	95% CI	*p*	*β*	95% CI	*p*	*β*	95% CI	*p*
IFN-γ												
Smoke	−0.60	−0.97/−0.23	**0.002**	−0.60	−0.98/−0.23	**0.002**	−0.67	−1.10/−0.24	**0.003**	−0.64	−2.09/−0.19	**0.006**
Snus	−0.36	−0.81/0.10	0.121	−0.39	−0.86/−0.08	0.106	−0.50	−1.02/0.33	0.066	−0.45	−1.00/0.11	0.111
Dual	−0.48	−0.93/−0.37	**0.034**	−0.53	−1.02/−0.04	**0.035**	−0.57	−1.12/−0.01	**0.045**	−0.55	−1.13/0.05	0.073
IL-10												
Smoke	−0.46	−0.77/−0.15	**0.004**	−0.45	−0.76/−0.14	**0.005**	−0.51	−0.89/−0.13	**0.009**	−0.50	−0.90/−0.10	**0.016**
Snus	−0.51	−0.89/−0.14	**0.008**	−0.50	−0.88/−0.11	**0.013**	−0.66	−1.13/−0.19	**0.007**	−0.66	−1.15/−0.17	**0.009**
Dual	−0.27	−0.64/0.10	0.156	−0.24	−0.64/0.17	0.250	−0.30	−0.79/0.19	0.226	−0.27	−0.79/0.25	0.309
TNF-α												
Smoke	−0.57	−0.89/−0.26	**<0.001**	−0.59	−0.90/−0.29	**<0.001**	−0.58	−0.92/−0.23	**0.001**	−0.55	−0.91/−0.19	**0.003**
Snus	−0.57	−0.96/−0.15	**0.003**	−0.53	−0.92/−0.15	**0.007**	−0.51	−0.94/−0.09	**0.019**	−0.45	−0.88/−0.01	**0.044**
Dual	−0.52	−0.90/−0.15	**0.007**	−0.48	−0.88/−0.08	**0.019**	−0.46	−0.90/−0.01	**0.045**	−0.47	−0.93/−0.01	**0.049**
IL-17a												
Smoke	−0.92	−1.51/−0.33	**0.003**	−0.87	−1.47/−028	**0.004**	−0.87	−1.61/−0.12	**0.024**	−0.83	−1.61/−0.05	**0.038**
Snus	−1.12	−1.84/−0.40	**0.003**	−0.97	−1.72/−0.28	**0.010**	−1.17	−2.09/−0.24	**0.014**	−1.13	−2.08/−0.19	**0.019**
Dual	−1.15	−1.86/−0.44	**0.002**	−0.91	−1.68/−0.13	**0.022**	−1.05	−2.01/−0.08	**0.034**	−1.09	−2.10/−0.08	**0.035**
IL-1β												
Smoke	−1.17	−1.84/−0.51	**0.001**	−1.21	−1.89/−0.54	**0.001**	−0.98	−1.81/−0.15	**0.021**	−0.89	−1.76/−0.01	**0.047**
Snus	−1.45	−2.27/−0.64	**0.001**	−1.52	−2.37/−0.68	**0.001**	−1.71	−2.73/−0.69	**0.001**	−1.64	−2.70/−0.58	**0.003**
Dual	−1.18	−1.99/−0.36	**0.004**	−1.31	−2.19/−0.43	**0.004**	−1.21	−2.27/−0.14	**0.027**	−1.10	−2.27/−0.01	**0.048**
IL-6												
Smoke	−0.28	−0.72/0.16	0.207	−0.27	−0.70/0.16	0.210	−0.25	−0.74/0.23	0.299	−0.26	−0.75/0.24	0.306
Snus	−0.57	−1.11/−0.03	**0.040**	−0.43	−0.97/0.11	0.114	−0.29	−0.89/0.30	0.330	−0.20	−0.80/0.40	0.503
Dual	−0.26	−0.79/0.27	0.343	−0.06	−0.62/0.50	0.838	−0.12	−0.74/0.51	0.714	−0.17	−0.81/0.47	0.603
IL-8												
Smoke	−0.61	−1.03/−0.19	**0.005**	−0.67	−1.08/−0.26	**0.001**	−0.48	−1.01/0.05	0.073	−0.55	−1.11/0.01	0.051
Snus	−1.07	−1.58/−0.55	**<0.001**	−1.09	−1.60/−0.58	**<0.001**	−0.93	−1.58/−0.28	**0.006**	−0.95	−1.62/−0.28	**0.006**
Dual	−0.83	−1.33/−0.32	**0.002**	−0.91	−1.44/−0.38	**0.001**	−0.88	−1.56/−0.20	**0.012**	−1.02	−1.74/−0.30	**0.006**

(Continues)

TABLE 4 (Continued)

	Unadjusted model			Model 1			Model 2			Model 3		
	β	95% CI	p	β	95% CI	p	β	95% CI	p	β	95% CI	p
IL-1ra												
Smoke	−0.41	−0.93/0.10	0.116	−0.42	−0.95/0.11	0.120	−2.25	−0.79/0.29	0.359	−0.31	−0.87/0.25	0.277
Snus	−0.68	−1.32/−0.05	**0.035**	−0.69	−1.35/−0.03	**0.041**	−0.35	−1.01/0.32	0.303	−0.32	−1.00/0.36	0.348
Dual	−0.26	−0.89/0.36	0.406	−0.27	−0.96/0.42	0.414	−0.17	−0.86/0.53	0.636	−0.30	−1.03/0.43	0.412
MCP-1												
Smoke	−0.13	−0.37/0.12	0.299	−0.17	−0.41/0.07	0.167	−0.10	−0.40/0.19	0.491	−0.14	−0.45/0.17	0.384
Snus	−0.44	−0.74/−0.14	**0.004**	−0.47	−0.77/−0.18	**0.002**	−0.49	−0.85/−0.12	**0.010**	−0.49	−0.87/−0.12	**0.011**
Dual	−0.13	−0.42/0.17	0.398	−0.20	−0.51/0.11	0.199	−0.18	−0.56/0.21	0.364	−0.26	−0.66/0.14	0.206

Note: Reference category: non-nicotine users. Model 1: Adjustment for sex and age; Model 2: Additional adjustment for somatic inflammatory illness, BMI, and low level of physical activity; Model 3: Additional adjustment for SDS and HSCL. *p*-values <0.05 are shown in bold.

others regarding chronic or acute illness or use of anti-inflammatory medication. Still, there may be factors not observed in this study affecting the inflammatory response differently between groups.

In conclusion, this study indicates that nicotine may have anti-inflammatory effects in the context of chronic, but currently abstinent, alcohol use. However, it is not a viable therapeutic option for reducing inflammation in individuals with AUD. Although nicotine may alleviate some of the negative consequences of alcohol use, it may also have other adverse effects and potentially increase the risk of further alcohol consumption. Our study indicates that nicotine may influence circulatory cytokine levels in alcohol-induced inflammation, but studies with larger sample sizes are needed to further investigate this association. Future studies of cytokine patterns in relation to mental or somatic health conditions are warranted to carefully investigate the effect of nicotine, by monitoring the use of any tobacco or nicotine product.

ACKNOWLEDGMENTS

We thank the participants of this study and the clinics, Blue Cross East, Riisby and Trasopp treatment centers, and the Blood donation center, Innlandet Hospital Trust, Hamar.

FUNDING INFORMATION

This work was financially supported by The Research Council of Norway, grant FRIPRO 251140.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest in this work.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Bolstad, I., Lien, L., Moe, J.S., Pandey, S., Toft, H. & Bramness, J.G. (2023) Inflammatory cytokines in alcohol use disorder patients are lower in smokers and users of smokeless tobacco. *Alcohol: Clinical and Experimental Research*, 00, 1–12. Available from: https://doi.org/10.1111/acer.15103