Alcohol 95 (2021) 45–50

Contents lists available at ScienceDirect

Alcohol

journal homepage: http://www.alcoholjournal.org/

Comparison of serum calprotectin, a marker of neutrophil activation, and other mediators of inflammation in response to alcohol consumption

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ARTICLE INFO

Article history:
Received 11 March 2021
Received in revised form
10 June 2021
Accepted 10 June 2021

Keywords:
biomarker
ethanol
immune dysfunction
leukocyte
liver

ABSTRACT

Aims: Previous studies have indicated that heavy alcohol intake stimulates inflammation and impairs the body's ability to regulate inflammation. The aim of this study was to compare changes in neutrophil calprotectin and a wide spectrum of other inflammatory mediators in response to heavy alcohol drinking.
Methods: Serum calprotectin (a marker of neutrophil activation), suPAR, CD163, and pro- (IL-6, IL-8, TNF-α) and anti-inflammatory (IL-10, TGF-β) cytokines were measured from 61 alcohol-dependent subjects (46 men, 15 women, mean age 43.6 ± 11.0 years) at the time of admission for detoxification and after 8 ± 2 days of abstinence. These biomarkers were also measured from age- and sex-matched healthy controls representing abstainers or light drinkers. The clinical assessments included detailed clinical interviews on the amounts and patterns of alcohol consumption and assays for biomarkers of alcohol consumption (GGT, CDT, MCV, GGT-CDT) and liver function (AST, ALT).
Results: The subjects with alcohol use disorder showed significantly higher concentrations of serum calprotectin ($p < 0.0005$), suPAR ($p < 0.01$), CD163 ($p < 0.01$), IL-6 ($p < 0.0005$), IL-8 ($p < 0.0005$), TNF-α ($p < 0.001$), and IL-10 ($p < 0.0005$) than healthy controls. These inflammatory mediators, except for CD163, remained elevated after the 8 ± 2-day period of supervised abstinence, which resulted in significant decreases in the biomarkers of alcohol consumption and indices of liver status. The AUC (0.855) for serum calprotectin in differentiating between the heavy drinkers and healthy controls was equal or equivalent with those of the conventional biomarkers of alcohol consumption (GGT:0.835 or CDT:0.803).
Conclusions: The data indicate that neutrophil calprotectin is released in response to heavy alcohol intake in a sensitive manner and may be associated with perpetuation of inflammation in patients with alcohol use disorder. Serum calprotectin may also prove to be a useful biomarker for inflammatory activity in alcohol-consuming patients.

Introduction

Excessive systemic inflammation and immune dysfunction have been suggested to play a pivotal role in the sequence of events leading to tissue injury in patients with alcohol use disorder (Adams, Conigrave, Lewohl, Haber, & Morley, 2020; Albillos, Lario, & Álvarez-Mon, 2014; Rehm et al., 2017; Szabo & Saha, 2015). Several types of inflammatory mediators in serum have been shown to change as a result of heavy alcohol consumption or alcohol-related liver disease (Achur, Freeman, & Vrana, 2010; Adams et al., 2020; Crews, Lawrimore, Walter, & Coleman, 2017; Homann et al., 1995; Latvala et al., 2005). Alcohol use also has an effect on cell-mediated immunity, including both the function and quantity of T cells (Li et al., 2019; Molina, Happel, Zhang, Kolls, & Nelson, 2010). Recent studies in experimental animal models

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https://doi.org/10.1016/j.alcohol.2021.06.001

Abbreviations

ALT	alanine aminotransferase
AST	aspartate aminotransferase
CD163	a biomarker of monocyte-macrophage activation
CDT	carbohydrate-deficient transferrin
GGT	gamma glutamyl transferase
IL	interleukin
suPAR	soluble urokinase plasminogen activator receptor, a biomarker of immune activation
TGF-β	transforming growth factor-beta
TNF-α	tumor necrosis factor-alpha

have suggested that the shifts in immune mediators may occur in an alcohol-dose dependent manner (Sureshchandra et al., 2019).

While a wide spectrum of new and emerging serum inflammatory biomarkers have recently been made available, as yet only limited information has been available on simultaneous comparisons of responses in the mediators of inflammation following heavy alcohol intake. Calprotectin comprises approximately 45% of the protein content in the cytosol of neutrophil granulocytes and is released upon neutrophil activation (Holmgaard et al., 2013; Nagareddy et al., 2013; Petersen et al., 2013; Pruenster, Vogl, Roth, & Sperandio, 2016; Shi et al., 2021; Vogl et al., 2007), which has been associated with both anti-infective and anti-inflammatory properties, including control of myelopoiesis, chelation of divalent cations, scavenging of reactive oxygen species, chemotaxis, and direct antimicrobial action (Holmgaard et al., 2013; Nagareddy et al., 2013; Petersen et al., 2013; Vogl et al., 2007). In addition, soluble urokinase plasminogen activator (suPAR) and CD163 (an endocytic receptor protein for haptoglobin-hemoglobin complexes) are proteins which have recently emerged as possible prospective risk factors for adverse clinical outcomes in inflammatory conditions (Andersen, Eugen-Olsen, Kofoed, Iversen, & Haugaard, 2008; Buehler et al., 2009; Koch et al., 2011; Møller, 2012; Thunø, Macho, & Eugen-Olsen, 2009). suPAR is expressed on many immunologically active cells, including monocytes, neutrophils, and activated T cells (Andersen et al., 2008; Koch et al., 2011; Thunø et al., 2009), whereas CD163 is found on macrophages and monocytes. Its serum levels increase in conjunction with the involvement of macrophages and free-radical induced tissue damage (Buehler et al., 2009; Møller, 2012).

To gain further insight on the changes in the status of inflammation and neutrophil activation in response to heavy alcohol intake, we compared the responses in serum calprotectin with those of a wide variety of other immune mediators, including suPAR, CD163, pro- (IL-6, IL-8, TNF-α) and anti-inflammatory (IL-10, TGF-β) cytokines and conventional biomarkers of alcohol consumption and liver status from alcohol-dependent subjects at the time of admission for detoxification and after an 8 ± 2 day period of supervised abstinence. For comparison, these parameters were also measured from age- and sex-matched control subjects representing apparently healthy abstainers or light drinkers.

Materials and methods

Participants

The main clinical and laboratory characteristics of the study subjects are summarized in Table 1. The study population included 61 patients with alcohol use disorder (46 men and 15 women) with a mean age 43.6 ± 11.0 years, who had been admitted for detoxification. Blood samples were collected at the time of admission and following an 8 ± 2-day period of supervised abstinence. All subjects were devoid of clinical and laboratory records indicating significant liver disease, comorbid substance abuse, major depression, inflammatory bowel diseases, or any immunological disorders. All these subjects showed a history of heavy alcohol drinking characterized by continuous consumption or repeated inebriations, the mean recent alcohol consumption being 110 grams/day, (range 50–520 grams/day) from the period of one month prior to sampling. The documentation of alcohol use was based on hospital records and detailed clinical interviews using a time-line follow-back technique that recorded data from the previous one month, one week, and past days preceding admission. The mean duration of abstinence prior to the first sampling time point was 2 ± 2 days. The study subjects volunteered for a follow-up with supervised abstinence during hospitalization. Blood alcohol concentrations during this period were measured by repeated analyses from breath air. The reference population consisted of apparently healthy age- and sex-matched individuals (43 men and 18 women, mean age 45.5 ± 13.6 years), who were either abstainers or light drinkers, whose daily ethanol consumption had not exceeded 40 grams on any occasion. All control subjects were also devoid of a history of recent illnesses or any immunological disorders.

All subjects gave their informed consent for the study. The protocol was approved by the local ethical committee and the study was conducted according to the provisions of the Declaration of Helsinki.

Laboratory methods

Serum was separated from blood samples by centrifugation (1500×*g* for 10 minutes) and stored at −70 °C prior to the measurements of the various biomarkers. Serum calprotectin concentrations were measured using the BÜHLMANN MRP8/14 ELISA kit according to the instructions of the manufacturer (BÜHLMANN Laboratories AG; Schönenbuch, Switzerland). Serum suPAR levels were measured using the suPARnostic enzyme-linked immunosorbent assay (ELISA) kit according to the instructions of the manufacturer (Virogates; Birkerød, Denmark). The measurements of CD163 were carried out using the Quantikine human CD163 ELISA assay (R&D Systems; Abingdon Science Park, United Kingdom). The concentrations of interleukins (IL-6, IL-8, IL-10) TNF-α, and TGF-β in serum were determined using Quantikine high sensitivity ELISA kits (R&D Systems; Abingdon Science Park, United Kingdom). Routine blood chemistry analyses were carried out using standard clinical chemical methods on an Abbott Architect c8000 automated clinical chemistry analyzer (Abbott Diagnostics, Abbott Laboratories; Abbott Park, Illinois, United States). Blood leukocyte counts were determined using a Sysmex XE-5000 automated Hematology Analyzer. All measurements were carried out in an SFS-EN ISO 15189:2013 accredited laboratory.

Table 1
Main clinical and laboratory characteristics of the study population.

	Heavy drinkers	Controls
n	61	61
Sex, male/female	46/15	43/18
Age, mean ± SD	43.6 ± 11.0	45.5 ± 13.6
GGT (U/L), median (IQR)	68.0 (36.5–159.5)***	22.0 (17.0–34.5)
CDT (%), median (IQR)	2.50 (2.0–4.4)***	1.84 (1.69–2.25)
ALT (U/L), median (IQR)	41.0 (25.5–65.0)***	26.0 (21.0–34.5)
AST (U/L), median (IQR)	39.0 (28.0–64.5)***	25.0 (21.0–29.3)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CDT, carbohydrate-deficient transferrin; GGT, gamma glutamyl transferase; IQR, interquartile range; U/L, unit/liter.
***$p < 0.001$ for comparison between the heavy drinkers and the control group.

Statistical methods

The values are reported as mean ± standard deviation (SD) or medians and interquartile ranges, as indicated. The levels of different mediators of inflammation were compared between the study groups using the Mann–Whitney U test. The comparisons between the different time points were made using paired samples *t* test, except for GGT, AST, and ALT, which were analyzed using Wilcoxon Signed Ranks Test due to skewed distributions of observations in these parameters. Spearman's rank correlation coefficient (r_s) was used for evaluating the correlations between the study variables. The area under the receiver operating characteristic (ROC) curve (AUC) was used to compare the predictive ability of various biomarkers in differentiating between the heavy drinkers and the control population. Positive likelihood ratio (LR+) and negative likelihood ratio (LR−) were calculated using the formulas: LR+ = sensitivity/(1−specificity) and LR− = (1−sensitivity)/specificity. A p value of <0.05 was considered statistically significant. Statistical analyses were carried out using IBM SPSS Statistics 24.0 (Armonk, New York, United States; IBM Corp.).

Results

Fig. 1 summarizes the levels of the various biomarkers of inflammatory status in the study groups. Heavy drinkers at the time of admission for detoxification showed significantly higher median values of serum calprotectin (4.15 vs. 1.02 μg/mL, $p < 0.0005$), suPAR (3.39 vs. 2.56 ng/mL, $p < 0.01$), CD163 (756 vs. 573 ng/mL, $p < 0.01$), interleukin-6 (IL-6) (3.27 vs. 1.35 pg/mL, $p < 0.0005$), IL-8 (22.4 vs. 13.8 pg/mL, $p < 0.0005$), TNF-α (1.20 vs. 0.31 pg/mL, $p < 0.001$), and IL-10 (0.72 vs. 0.51 pg/mL, $p < 0.0005$) than those in the healthy controls. During the follow-up with supervised abstinence there was a significant decrease in biomarkers of alcohol consumption and liver dysfunction – GGT (median decrease −21.0 [IQR −66.0 to −4.5] U/L, $p < 0.0005$), MCV (mean decrease −3.93 [95% CI 5.92 to −1.95] fL, $p < 0.0005$), AST (median decrease −11.0 [IQR −30.5 to −4.0] U/L, $p < 0.001$) and ALT (median decrease −6.0 [IQR −25.0 to 3.0] U/L, $p < 0.01$). Among the mediators of inflammation, a significant decrease only in the level of CD163 was noted (mean decrease −117 [95% CI −205 to −29.0] ng/mL, $p < 0.02$), whereas the levels of serum calprotectin, suPAR, IL-6, IL-8, TNF-α, and IL-10 concentrations remained significantly higher than those in the healthy controls (Fig. 1).

The discriminative power and likelihood ratios of the various biomarkers in distinguishing between the heavy drinkers and the control group are summarized in Table 2. The sensitivity (62.3%) and specificity (88.5%) (AUC = 0.855) of serum calprotectin in correctly classifying the heavy alcohol drinkers was markedly higher than that in the other mediators of inflammation, and interestingly, even comparable to the diagnostic accuracy of the actual biomarkers of alcohol consumption (Table 2).

At the time of admission, the levels of ethanol consumption from the past one month prior to blood sampling correlated significantly with serum GGT ($r_s = 0.333$, $p < 0.02$), GGT-CDT ($r_s = 0.413$, $p < 0.01$), AST ($r_s = 0.356$, $p < 0.01$), ALT ($r_s = 0.290$, $p < 0.05$), and serum ferritin ($r_s = 0.373$, $p < 0.01$), whereas not with the other markers. Serum calprotectin showed statistically significant correlations with blood leukocyte counts ($r_s = 0.612$, $p < 0.001$) and suPAR ($r_s = 0.476$, $p < 0.001$) levels, whereas no significant correlations were observed in comparisons with calprotectin and the other mediators of inflammation or biomarkers of alcohol consumption and liver status. In the follow-up samples, calprotectin levels correlated with blood leukocytes ($r_s = 0.541$, $p < 0.001$), suPAR ($r_s = 0.377$, $p < 0.01$), and in addition, with IL-6 ($r_s = 0.538$, $p < 0.001$), IL-8 ($r_s = 0.538$, $p < 0.001$) levels.

In further analyses of the associations between the current biomarkers and blood leukocytes, a cellular biomarker of inflammation, and serum ferritin, an acute phase reactant of inflammation,

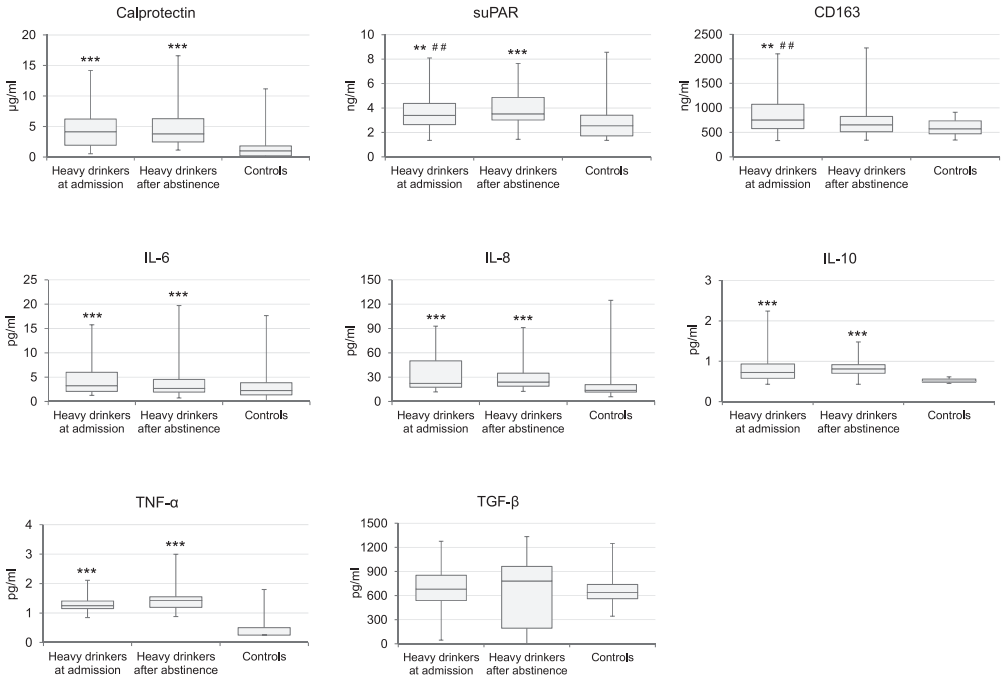

Fig. 1. Mediators of inflammation in alcohol-dependent subjects at the time of admission and after supervised abstinence and in healthy controls. The values are shown as medians and interquartile ranges. **$p < 0.01$; ***$p < 0.001$ for comparisons between the heavy drinkers at the time of admission and healthy controls; ##$p < 0.01$ for comparisons with the heavy drinkers after abstinence and healthy controls. suPAR, soluble urokinase plasminogen activator receptor; CD163, a biomarker of monocyte-macrophage activation; IL, interleukin; TNF-α, tumor necrosis factor-alpha; TGF-β, transforming growth factor-beta

Table 2
Characteristics of the biomarkers in distinguishing between healthy controls and heavy drinkers.

Biomarker	Cut-off Male/Female	Sensitivity %	Specificity %	AUC	*p**	LR+	LR−
Calprotectin	3.0 μg/mL	62.3	88.5	0.855		5.4	0.4
suPAR	4.0 ng/mL	29.8	88.0	0.706	<0.05	2.5	0.8
CD163	840 ng/mL	41.5	96.0	0.720	<0.05	10.4	0.6
IL-6	5.4 pg/mL	32.7	85.0	0.764		2.2	0.8
IL-8	46 pg/mL	27.6	95.1	0.773		5.6	0.8
IL-10	0.6 pg/mL	66.7	94.1	0.849		11.3	0.4
TGF-β	890 pg/mL	20.0	95.1	0.543	<0.0005	4.1	0.8
TNF-α	1.59 pg/mL	15.0	95.7	0.870		3.5	0.9
GGT	60/40 U/L	62.3	93.4	0.835		9.5	0.4
CDT	2.5%	50.0	91.3	0.803		5.8	0.5
GGT-CDT	4.3/3.8	81.6	100.0	0.916		>20[#]	0.2
ALT	50/35 U/L	41.0	85.2	0.676	<0.001	2.8	0.7
AST	45/35 U/L	37.7	91.8	0.768		4.6	0.7

*p values indicate the significances of differences in AUCs, as compared to serum calprotectin.
[#]exact LR + value cannot be determined, since specificity is 100%.

significant correlations were noted with blood leukocyte counts and suPAR ($r_s = 0.398$, $p < 0.01$) and IL-6 ($r_s = 0.378$, $p < 0.01$). Serum ferritin levels were found to correlate with suPAR ($r_s = 0.372$, $p < 0.01$), CD163 ($r_s = 0.611$, $p < 0.001$), IL-8 ($r_s = 0.516$, $p < 0.001$), GGT ($r_s = 0.722$, $p < 0.001$), GGT-CDT ($r_s = 0.632$, $p < 0.001$), ALT ($r_s = 0.714$, $p < 0.001$), and AST ($r_s = 0.745$, $p < 0.001$). Significant correlations between AST, a biomarker of liver status, and the biomarkers of immune status were noted with suPAR ($r_s = 0.389$, $p < 0.01$), CD163 ($r_s = 0.683$, $p < 0.001$), IL-6 ($r_s = 0.299$, $p < 0.05$), and IL-8 ($r_s = 0.508$, $p < 0.005$).

Discussion

Our study comparing serum calprotectin, a marker of neutrophil activation, with a wide spectrum of other biomarkers of inflammation following heavy alcohol intake demonstrates distinct characteristics in the patterns of alcohol-induced changes in the immune system. The highly sensitive responses in calprotectin release indicate a potent role of heavy drinking as an inducer of neutrophil-driven inflammation, which, based on previous observations from other clinical conditions, may associate with significant inflammatory processes and the need for scavenging of reactive oxygen species (Holmgaard et al., 2013; Kalla et al., 2016; Petersen et al., 2013; Shi et al., 2021). In accordance with the above view, recent studies from both healthy volunteers and alcoholic patients have indicated that even a single alcohol binge may have an impact on neutrophil function and may also cause an increase in acute phase protein levels in circulation (Bala, Marcos, Gattu, Catalano, & Szabo, 2014; Li et al., 2017; Stadlbauer et al., 2019).

The data further suggest that serum calprotectin could serve as a sensitive biomarker for the inflammatory stimuli created by heavy alcohol intake. Indeed, the data indicate that with two-thirds of the heavy alcohol drinkers showing elevated serum calprotectin levels, the diagnostic accuracy of this biomarker in distinguishing between the healthy controls and heavy drinkers is comparable to that of the well-established markers of heavy alcohol intake, such as GGT or CDT. Thus, future studies appear warranted to further explore the clinical utility of serum calprotectin assays also being used in routine patient management as a possible biomarker of alcohol-induced inflammatory responses.

Previous studies have proposed that calprotectin could play a physiological role in controlling inflammation and leukocyte trafficking, which is needed to yield more coordinated and appropriate immune reactions for preventing tissue damage (Kerkhoff, Klempt, & Sorg, 1998; Nagareddy et al., 2013; Petersen et al., 2013; Vogl et al., 2007). A switch of the immune system toward either the pro- or anti-inflammatory direction may be influenced by multiple precipitating factors, such as the prevailing metabolic status in the body and associated changes in extracellular pH (Nagareddy et al., 2013). While the present observations show simultaneous changes in multiple mediators of inflammation with both pro- and anti-inflammatory characteristics, the question of whether such responses represent protective or damaging effects in alcohol-exposed tissues remains, however, unclear. Parallel responses could reflect an attempt to regulate tissue damage. Recently, IL-6, which has generally been regarded as a pro-inflammatory cytokine, has also been linked with anti-inflammatory properties and induction of hepatoprotective genes (Kawaratani et al., 2013; McGinnis et al., 2015; Muñoz-Cánoves, Scheele, Pedersen, & Serrano, 2013; Tanaka & Kishimoto, 2014). IL-6 response may also be associated with the regulation of acute phase responses and tissue regeneration (Niemelä, Kangastupa, Niemelä, Bloigu, & Juvonen, 2016; Tanaka & Kishimoto, 2014). Both IL-6 and IL-10 cytokines can stimulate the Th2 pathway, activate anti-inflammatory cascades, and inhibit TNF-α (McGinnis et al., 2015; Vidali et al., 2008). On the other hand, we observed notable elevations in circulating levels of IL-8 and TNF-α, which are potent pro-inflammatory mediators of tissue damage and oxidative stress (Ernandez & Mayadas, 2009; Neuman et al., 2001; Qazi, Tang, & Qazi, 2011; Vidali et al., 2008). They also have the ability to attract neutrophils and regulate macrophage production.

Although in the present series we did not find significant correlations between the quantities of self-reported alcohol intake and the components of the immune system, it should be noted that, based on recent observations in experimental animal models, the transcriptional and functional responses of peripheral blood mononuclear cells following chronic alcohol drinking may take place in an alcohol-dose dependent manner (Sureshchandra et al., 2019). Quantitative estimates of alcohol drinking in human studies based on self-reports are obviously vulnerable to the shortcomings of this memory-dependent channel of information and therefore, further studies are clearly warranted to examine the dose-effect relationships between alcohol intake and immune status utilizing, in addition, novel biomarkers of alcohol consumption, such as ethylglucuronide in urine, to get more precise estimates of recent drinking.

In patients with alcohol-induced liver disease, the balance between pro- and anti-inflammatory cytokines has previously been shown to be highly skewed toward overwhelming production of pro-inflammatory mediators (Crews et al., 2017; Kawaratani et al., 2013; Latvala et al., 2005). This may be due to excessive antigen loading from ethanol metabolites, gut-derived endotoxin, and bacterial products, as well as impaired anti-inflammatory capacity

(Freeman et al., 2005; Kawaratani et al., 2013; Lowe et al., 2017). The pro-inflammatory status in such patients may further trigger the production of reactive oxygen species, oxidative stress, and release of immune mediators (Devries et al., 2008; di Penta et al., 2013; Homann et al., 1995; Lipscey, Hanslin, Stålberg, Smekal, & Larsson, 2019; Vidali et al., 2008). It is also noteworthy that alcoholics with recent drinking have been shown to present with higher levels of circulating neutrophils, which coincides with increased serum liver enzyme activities, liver inflammation, and upregulation of pro-inflammatory cytokines (Bertola, Park, & Gao, 2013; Cai et al., 2017; Li et al., 2017). Based on the above observation and current observations, it appears that a coordinated balance between pro- and anti-inflammatory cascades of inflammation is of crucial importance in counteracting alcohol-induced inflammatory threat and the development of tissue damage.

Alcohol-dependent subjects also showed profound responses in suPAR and CD163, further supporting the view that heavy alcohol use readily induces disease-associated mediators of inflammation. suPAR is found on various immunologically active cells and has been previously shown to be elevated in patients with severe systemic inflammation (Andersen et al., 2008; Koch et al., 2011; Thunø et al., 2009; Tuomi et al., 2014). This biomarker has also been proposed as a predictor of mortality in various clinical conditions, showing an association with poor disease outcomes (Andersen et al., 2008; Casagranda et al., 2015; Eugen-Olsen, 2011; Eugen-Olsen et al., 2010; Koch et al., 2011; Rohde, Polcwiartek, Andersen, Vang, & Nielsen, 2018; Thunø et al., 2009). CD163 is present on macrophages and circulating monocytes and has been implicated as an independent risk marker of disease progression and fibrogenesis in inflammatory diseases (Møller, 2012; Rødgaard-Hansen et al., 2017). It may also play a role in innate immune defense by sequestering hemoglobin-bound iron and scavenging of oxidative stress-induced by-products (Buehler et al., 2009; de Couto et al., 2015).

Further studies appear warranted to examine whether the use of current biomarkers of immune dysfunction could be of value in long-term follow-up of immune function and as predictors of outcome in individuals engaged in frequent episodes of heavy alcohol drinking. Most mediators of inflammation seem to remain elevated and stable at least during a relatively short period of abstinence. Previous studies have observed that clinical deterioration, which is frequently observed among alcohol-dependent patients following hospitalization and cessation of ethanol intake, may be mediated by immunological mechanisms (Marshall, Burnett, Zetterman, & Sorrell, 1983). It may be assumed that skewed balances in the ratios of pro- and anti-inflammatory cytokines correlate with unfavorable over-activation of the sympathetic nervous system, production of reactive oxygen species, and oxidative stress, which lead to immunocompromised status and induction of both local and systemic inflammation (Costa, Snipe, Kitic, & Gibson, 2017; Devries et al., 2008; di Penta et al., 2013; Dimitrov, Hulteng, & Hong, 2017; Niemelä et al., 2016; Szabo & Saha, 2015). The extent of changes in the mediators of inflammation may also be important in determining the status of individual susceptibility to coincidentally occurring pathogens. A prolonged pro-inflammatory milieu could also play a pathogenic role in the development of endotoxemia, increased gastrointestinal permeability, and increased risk of mortality in alcoholic patients with bacteremia (Costa et al., 2017; Huang, Chen, & Hsu, 2020; Linderoth, Jepsen, Schønheyder, Johnsen, & Sørensen, 2006).

Taken together, the present data demonstrate distinct changes in the status of inflammation as a result of heavy alcohol drinking. Current data on serum calprotectin responses also suggest an important role for neutrophil function in perpetuating inflammation in patients with alcohol use disorder. Future work is required to address the hypothesis whether such changes could influence the susceptibility of heavy alcohol users to viral and bacterial infections, sterile inflammation, or tissue damage (Szabo & Saha, 2015). Additional research is also needed to address the questions of how and to what extent modifications of lifestyle and associated modulation of immune responses could help as part of therapeutic and preventive strategies in reduction of inflammation and oxidative stress status in alcohol consumers (Costa et al., 2017; Devries et al., 2008; Niemelä et al., 2016; Oh et al., 2015).

Funding

This work was supported in part by Competitive State Research Financing of the Expert Responsibility area of Seinäjoki Central Hospital and University of Tampere, VTR 6100/3243, the Finnish Foundation for the Promotion of Laboratory Medicine, and the Finnish Society of Clinical Chemistry.

Authors' contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

The expert assistance of Ms. Anni Halkola and Mrs. Taana Sandman is gratefully acknowledged.

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