Published in final edited form as:
J Neuroimmune Pharmacol. 2010 March ; 5(1): 83–91. doi:10.1007/s11481-009-9185-z.

Circulating Cytokines as Biomarkers of Alcohol Abuse and Alcoholism

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Abstract

There are currently no consistent objective biochemical markers of alcohol abuse and alcoholism. Development of reliable diagnostic biomarkers that permit accurate assessment of alcohol intake and patterns of drinking is of prime importance to treatment and research fields. Diagnostic biomarker development in other diseases has demonstrated the utility of both open, systems biology, screening for biomarkers and more rational focused efforts on specific biomolecules or families of biomolecules. Long term alcohol consumption leads to altered inflammatory cell and adaptive immune responses with associated pathologies and increased incidence of infections. This has led researchers to focus attention on identifying cytokine biomarkers in models of alcohol abuse. Alcohol is known to alter cytokine levels in plasma and a variety of tissues including lung, liver, and very importantly brain. A number of cytokine biomarker candidates have been identified, including: TNF alpha, IL1-alpha, IL1-beta, IL6, IL8, IL12 and MCP-1. This is an emerging and potentially exciting avenue of research in that circulating cytokines may contribute to diagnostic biomarker panels and a combination of multiple biomarkers may significantly increase the sensitivity and specificity of the biochemical tests aiding reliable and accurate detection of excessive alcohol intake.

Keywords

alcoholism; alcohol abuse; biomarkers; cytokines; diagnostics

Introduction

Alcohol abuse and alcoholism remain serious societal problems in the US and the rest of the world. The lack of reliable diagnostic criteria and markers is a major obstacle to the diagnosis, treatment, and research of alcohol abuse and alcoholism. This review will address the definition of biomarkers, and the types of biomolecules used as biomarkers. The potential uses and previously proposed biomarkers in alcohol-related disorders are then discussed. Lastly, the potential use of cytokines as biomarkers of alcohol intake and immune dysfunction is considered.

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Disclaimers: None

Alcohol consumption levels directly relate to the behavioral aspects of alcoholism. Efficient and accurate clinical tests for alcohol consumption, typology, and alcohol-induced disorders are critically needed in alcoholism and alcohol abuse treatment and research. Although meticulous efforts have been made to construct interview formats such as Alcohol Use Disorders Identification Test Consumption (AUDIT-C) (Bradley et al., 2007;Bush et al., 1998), and CAGE (Ewing, 1984) that quantify alcohol intake, there are serious limitations associated with these approaches. This is particularly true in cases where individuals are forced to deny or minimize the magnitude of drinking behavior in order to mitigate personal, professional, or legal ramifications of alcohol abuse (Pernanen, 1974;Fuller et al., 1988). Thus, it is clear that a measurable clinical biomarker test that could provide an objective assessment of drinking intake/behavior would alleviate the uncertainties of currently available reporting methods. Furthermore, novel clinical diagnostics that identify alcohol-induced tissue damage could improve clinical care and have applications in alcohol abuse treatment.

What is a biomarker?

The discussion of biomarker diagnostics in alcohol abuse and alcoholism begins with a definition of what a biomarker is and the potential uses of biomarker diagnostics. The NIH Biomarkers Definitions Working Group has defined a biomarker strictly as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention” (Biomarkers Definitions Working Group, 2001). The critical aspect of this definition is that a biomarker is indicative of a specifically-defined state. While the term biomarker is often misused in reference to any difference in biomolecule level means between two populations, this is a misapplication of the term because such a difference in mean levels may not be sufficiently robust to diagnose the disease in individual patients. We also suggest the use of the term biomarker to refer only to validated biomarkers while biomolecules that show promise as biomarkers but remain to be validated be referred to as biomarker targets.

Types of biomolecules used as biomarkers

The types of biomolecules that may serve as the best biomarkers of alcohol intake behavior and subsequent alcohol-induced tissue injury are not known. Macromolecules such as nucleic acids, proteins, or protein adducts could all be potential markers. Alternatively, small molecule or non-biochemical tests such as *in vivo* imaging could serve as a potential biomarker. In this regard, numerous studies have focused their attention on biomarkers that could be effectively used for alcohol abuse and alcoholism. Previous studies in this direction provided valuable examples of technologies that can be used in alcohol research based on the analysis of nucleic acids (Biermann et al., 2007;Walker and Grant, 2006), proteins (Freeman et al., 2006;Nomura et al., 2007), or small molecules (Bradford et al., 2008;De et al., 2007;Stephanson et al., 2007). Irrespective of the nature of the molecule or test, the best biomarker is the one that can effectively diagnose drinking behavior. Thus, while it may be appealing to think of a biomarker as a single molecule (e.g., an mRNA or a protein), increasing evidence suggests that a panel of molecules in combination may function as the best biomarkers with regard to sensitivity and specificity as shown in the case of lung cancer (Spira et al., 2007), Alzheimer’s disease (Ray et al., 2007) and amyotrophic lateral sclerosis (ALS) (Mitchell et al., 2009). Indeed, this approach has begun to be tested in alcohol diagnostics (Anton et al., 2002;Rinck et al., 2007).

Potential uses of biomarkers in alcohol-related disorders

With a biomarker defined as an indicator of a specific state, the next step for those in the alcohol research field is to define what state(s) is (are) being classified. One major goal of alcohol biomarker development is the generation of clinical diagnostics for identification of alcoholism. A biomarker of alcoholism is not strictly possible as DSM-IV criteria are based

on the behavior of the patient and classify patients into alcohol abusing and alcohol dependent categories (Table 1). However, biomarkers may be used to define alcohol intake levels as an indicator of alcohol use and potentially abuse. Therefore, biomarkers may be used to identify patients with historically high alcohol intake levels who are likely to then be diagnosed as alcohol abusing or alcohol dependent by the standard DSM-IV psychological criteria. Another potential use of biomarkers is in identifying alcohol-induced tissue damage. Biomarkers developed for this purpose could be used as diagnostics of specific complications such as cardiovascular disease, liver disease, central and peripheral nervous system damage, and immune deficiency. Whatever the state intended to be indicated/diagnosed by a biomarker, metrics of a biomarker's performance, such as sensitivity and specificity as a diagnostic are needed.

A potential source of confusion in the field is that biomarkers of alcohol consumption may relate to, or even include, some of the same specific molecules as biomarkers of alcohol-induced tissue damage and vice versa. It is important to again remember that a biomarker is defined by its ability to classify a particular state. Biomarkers of high alcohol intake levels may originate with alcohol-induced tissue damage, but are optimized to detect a level of drinking, while a biomarker for tissue damage will be selected to most accurately diagnose a certain type of tissue damage. Therefore, biomarkers are developed to achieve different outcomes or measure different (and selective) states.

Successful development of reliable biochemical biomarkers that permit accurate assessment of alcohol intake, drinking patterns, or tissue damage would have obvious and widespread utility. One emerging and exciting avenue of research is the use of plasma or serum protein levels, particularly cytokines, as potential biomarkers of alcoholism and alcohol-induced tissue damage. As noted in Figure 1, the circulation represents a non-invasive window on drinking pathology. Alcohol consumption can lead to alterations in cytokine production and release from all compartments of the body. Growing evidence suggests that these circulating cytokines can directly affect behavior and mood. In the present context, however, they may also serve as potential biomarkers of drinking behavior and alcohol-induced tissue damage. We, herein, develop the hypothesis that circulating cytokines may contribute to diagnostic biomarker panels. The combination of multiple biomarkers may significantly increase the sensitivity and specificity of the biochemical tests aiding early and accurate detection.

The desire for serum or plasma protein biomarkers of health and disease is not a novel concept and has been applied clinically for many years in ELISAs and other tests. Proteomics as a tool for biomarker discovery remains relatively new (Xiao et al., 2005) however biomarker discovery is a different question than the simple determination of a differentially expressed protein in the disease state versus the control state. A usable biomarker must be both sensitive (% of diseased subjects with marker) and specific (% of non-diseased subjects without the marker) in a population with normal prevalence. The majority of reports on proteomic discovery of serum biomarkers have been in the cancer field (Shau et al., 2003). We have chosen to classify protein biomarkers into three potential types: binary, single, and pattern. Binary biomarkers are the simplest and consist of a protein that is only present in either the diseased or control state; an on/off state with disease. Single biomarkers are single proteins that change in abundance with disease. A number of biomarkers such as this exist (e.g., prostate specific antigen, PSA). Pattern biomarkers, on the other hand, represent the use of multiple proteins for determination (Harasymiw and Bean, 2001;Javors and Johnson, 2003) and offer the promise of more rigorous detection.

Current biomarkers of alcoholism

The search for the development of diagnostic tools of alcohol abuse is not new. There has been significant research progress in this area to develop diagnostics of alcohol intake with a notable success being in directly measuring acute alcohol intake. A number of accurate methods for determining blood alcohol concentrations (BAC) through breath and blood now exist. This technology has developed to the point that small and inexpensive instruments are currently being used by thousands of law enforcement, medical, and security personnel.

The literature on potential plasma, blood, urine, and hair biomarkers has been thoroughly reviewed elsewhere (Conigrave et al., 2003;Conigrave et al., 2002;Das et al., 2008;Hannuksela et al., 2007;Helander, 2003). At present, the most common laboratory tests for alcohol intake include: gamma-glutamyltransferase (GGT) (Taracha et al., 2001), mean corpuscular volume (MCV) (Koch et al., 2004), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Niemela, 2007), sialylation of apolipoprotein J (SIJ) (Ghosh et al., 2001), carbohydrate-deficient transferrin (CDT) (Golka and Wiese, 2004;Hock et al., 2005), ethyl glucuronide (EtG) (Kissack et al., 2008), 5-hydroxytryptophol (5HTOL) (Helander and Eriksson, 2002), Phosphatidylethanol (PEth) (Comasco et al., 2009;Hansson et al., 1997), and homocysteine (Bleich and Hillemacher, 2009), are small molecule markers of alcohol intake. A detailed quantitative comparison of specificity, sensitivity and usefulness of these different biomarkers is beyond the scope of this review. However, in a broad sense, the utility of these biomarkers as diagnostic tools is greatly hampered due to variable results in different populations and low sensitivity and specificity. For example, clinical studies of CDT have found a range of sensitivities (77–100%) and specificities (10–85%) (Berner et al., 2006;Koch et al., 2004;Miller et al., 2006). These limitations may arise from several factors. 1) Most of these biomarkers have been proposed as unitary measures; however, combining multiple markers has been demonstrated to be more effective, suggesting that a panel of markers may result in better sensitivity and specificity (Anton et al., 2002;Korzec et al., 2005;Rinck et al., 2007;Korzec et al., 2009). 2) Many biomarkers relate to hepatic function, which is well known to be altered with alcohol abuse. Hepatic function is also impaired, however, in a number of other conditions that leads to false positives and reduced specificity. 3) Certain biomarkers may be detectable only during high alcohol intake or in conjugation with co-morbidities. In light of these limitations, together with other variations such as geographic and nutritional factors, there will be a higher level of false negative data and reduced sensitivity. While all of the biomarkers currently implicated have utility, they have not been universally accepted nor generally adopted in clinical practice. Thus, a need for more robust and dependable biomarkers of alcoholism is required and the current review examines one such group of potential biomarkers - the cytokines.

Cytokines as clinical biomarkers in other neurological disorders

In the last decade, a number of studies have examined the potential utility of plasma or CSF cytokines as disease diagnostics in neurological disorders as case studies of how alcohol research could potentially can benefit from cytokine profiling. An excellent review has previously summarized the potential use of cytokine biomarkers in various CNS disorders such as stroke, epilepsy, Parkinson's disease (PD), Alzheimer's disease (AD) and other neurological disorders (Lucas et al., 2006). Elevated levels of various cytokines in the course of CNS disorders have also been implicated from the point of view of potential therapeutic intervention (Perry, 2004). In epilepsy studies, an increase in serum cytokine levels has been documented, including alterations in TNF-alpha, IFN-gamma, IL-1beta, IL-2, IL-4, and IL-6 as compared to controls (Bauer et al., 2009). CSF analysis was also carried out indicating the presence of IL-6, IL-1, IL-2, IL-4, and IFN-gamma. Thus, increased post-ictal serum cytokine levels in

patients with seizure disorders could be used as biomarkers for detection (Sinha et al., 2008), which is in agreement with earlier studies (Lucas et al., 2006).

In an effort to have molecular and biochemical markers of AD and to complement clinical approaches for early and accurate detection, levels of chemokines and cytokines in the serum and CSF of patients have been measured (Choi et al., 2008). The results indicate that MCP-1 was the only cytokine detectable in CSF, and that its levels did not differ between control and disease groups. The data indicate that immunological responses are not major contributors to the pathogenesis of AD and PD (Choi et al., 2008). However, in the case of PD, cytokines such as IL-1, TNF-alpha, IL-6 have been previously implicated although there is considerable controversy [as discussed by (Lucas et al., 2006)]. A landmark study identified a panel of plasma proteins, including a number of cytokines that serves as an accurate diagnostic of AD with ~90% accuracy (Ray et al., 2007). Recently, clinical studies using CSF from amyotrophic lateral sclerosis (ALS) patients have shown the existence of distinct biomarker profiles. Higher expression of IL-8 was found to be associated with a group of patients with lower levels of physical function. A CSF inflammatory profile analysis of cytokine and growth factors may distinguish patients with ALS from other neurologic disease controls, and has been suggested as a biomarker panel to aid in diagnosis (Mitchell et al., 2009).

These reports clearly document the potential use of cytokine markers in developing biomarker panel signatures of neurological disease. Inflammation is not only involved in acute CNS conditions such as stroke and traumatic injury, but also may be a key factor in chronic and neurodegenerative conditions such as AD, PD, and ALS. The availability and promise of effective treatments for neurodegenerative disorders is increasing the importance for early diagnosis through novel highly sensitive biomarkers such as cytokines. These results have led us to focus our attention towards identifying cytokine biomarkers in models of alcohol abuse. Taking these findings of cytokine profiling in these CNS diseases as a model, there is a strong rationale for examining cytokines as biomarkers of alcohol intake and alcohol induced tissue damage as well.

Consequences of alcoholism on the host immune system

Low levels of ethanol are commonly consumed as part of normal daily behavior. It is commonly accepted that moderate amounts of polyphenol rich beverages such as beer or wine may have beneficial health effects including on the immune system. On the other hand, high doses of alcohol consumption can directly suppress a wide range of immune responses, and alcohol abuse is associated with an increased incidence of a number of infectious diseases (Romeo et al., 2007).

Alcohol intake has been shown to play a pivotal role in alterations of innate immune responses. Individuals consuming alcohol have been reported to demonstrate a delayed and impaired hypersensitivity response (Tonnesen et al., 1992). The same observations of delayed response have also been made in a mouse model of chronic alcohol administration (Jayasinghe et al., 1992). It has also been reported that severe alcohol intake inhibits the monocyte-derived myeloid Dendritic Cell’s (DCs) capacity for inducing activation of T-cells in humans. Moreover, alcohol-treated DCs showed reduced IL-12, increased IL-10 production, and a decrease in expression of the co-stimulatory molecules CD80 and CD86 (Mandrekar et al., 2004;Szabo et al., 2004). Decreased production of IL-6, IL-12, IL-17A, IFN-gamma and increased levels of IL-13 cytokines as a result of ovalbumin stimulation in alcohol-consuming mice have also been observed (Heinz and Waltenbaugh, 2007). The data indicate that ethanol alters CD11c+/CD8(alpha)+ DC function, affecting the cytokines responsible for adaptive immune responses.

The effects of different levels of alcohol consumption as well as type of alcoholic beverage on the activity, number, distribution, balance, interaction and response of immunocompetent cells have been extensively reviewed (Diaz et al., 2002;Szabo, 1999). In total, alcohol consumption leads to altered inflammatory cell and adaptive immune responses and to increased incidence of infections and during infection, poor presentation of infectious agents and other organ-specific immune-mediated effects (Szabo and Mandrekar, 2009). Furthermore, this altered immune response may be observed through measurements of plasma/serum cytokine levels.

Cytokine association with alcohol induced pathologies

Cytokines are a class of multifunctional proteins that are implicated in cellular communication and activation. Cytokines are critical to the development and functioning of both innate and adaptive immune response, and not just limited to the immune system, but also involved in developmental processes. The cytokines could be of type Th1 (proinflammatory) or Th2 (anti-inflammatory) depending upon their role in the immune system. Cytokines impact tissues in a complex manner that regulates inflammation, cell death, cell proliferation, cell migration, and healing mechanisms. Alcohol is known to alter cytokine levels in a variety of tissues including plasma, lung, liver, brain (Crews et al., 2006a). These alterations could contribute to changes in the central nervous system leading to long-term changes in behavior and neurodegeneration (Figure 1). These studies suggest that measurement of cytokines could be a key course of action in understanding alcohol neural pathogenesis. As described below, much of the literature to date has focused on alcohol-induced liver disease (Table 2). These studies highlight the importance of examining cytokine changes across the time course of alcoholism to understand the initial changes directly resulting from alcohol use and the secondary pathology that occurs with late stage alcohol-induced tissue damage. The relationship between cytokines and the metabolic consequences of chronic alcoholism has been studied since the early 1990s. Circulating cytokines such as tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1) and IL-6 are found to be elevated in both chronic and acute alcohol-induced liver disease. These have been primarily correlated with the metabolic consequences and abnormalities of liver injury due to alcohol intake (Khoruts et al., 1991). The concentrations of all three cytokines have been correlated with biochemical parameters of liver injury, hepatic protein synthesis and serum IgG concentration.

Recently, the measurement of serum TNF-alpha levels has become routine in clinical practice and it has been shown that TNF-alpha contributes to alcohol-induced organ dysfunction, including alcoholic hepatitis. Serum levels of TNF-alpha levels have been demonstrated to be higher in hospitalized alcoholics than in the general population, regardless of alcohol consumption level (Gonzalez-Quintela et al., 2008). Furthermore the highest TNF-alpha levels, were correlated with liver dysfunction. It is important to note that light-to-moderate drinking had no significant effect on the levels of serum TNF-alpha levels (Gonzalez-Quintela et al., 2008).

In a recent study, alcoholic patients were examined for the production of cytokines by peripheral blood (PB) monocytes and this was related to the amount of alcohol intake, as well as liver disease (Laso et al., 2007). As shown in Table 2, a significant increase in spontaneous production of IL1-beta, IL6, IL12, and TNF-alpha was observed in chronic alcoholics without liver disease compared with those who are alcoholic with liver cirrhosis. On the other hand, chronic alcoholics with liver disease still consuming alcohol showed abnormally low production of IL1-beta and TNF-alpha (Laso et al., 2007).

In addition to liver injury, there is a growing understanding of the role of cytokines in alcohol-induced neurodegeneration. In a rat model, alcohol-induced neurodegeneration is shown to be due to neuronal death during intoxication and is related to increased oxidative stress and

proinflammatory proteins that are neurotoxic (Crews and Nixon, 2009). Degeneration is associated with increased NF-kappaB proinflammatory transcription and decreased CREB transcription. However, abstinence after binge ethanol intoxication results in neurogenesis and regeneration of brain structure and function (Crews and Nixon, 2009). Studies using animal models have documented the effects of alcohol on acute adolescent neurogenesis by alcohol (Crews et al., 2006b). Studies on human postmortem brains from alcoholics have shown increased concentrations of monocyte chemoattractant protein 1 (MCP-1) and microglial activities that may have contributed to the alcohol-induced pathogenesis (He and Crews, 2008).

The interactions between altered levels of cytokines in alcoholics and infectious agents provide mechanistic insight into alcohol pathobiology. (Molina et al., 2006)have studied the effect of chronic alcoholism on nutritional, metabolic and immune alterations during the initial 10-month asymptomatic phase of SIV infection in nonhuman primate rhesus macaques. Chronic alcohol/SIV(+) animals showed a higher viral load at 3 months post-SIV infection, as well as muscle TNF-alpha mRNA expression was markedly increased (Molina et al., 2006).

Interestingly, there are also reported associations between cytokine gene polymorphisms and predisposition to alcoholism. Marcos and colleagues reported that an IL-10 gene polymorphism is associated with alcoholism and not with alcoholic liver disease as a result of alcoholism (Marcos et al., 2008). This study in Spanish subjects demonstrated that the frequency of allele A carriers (CA and AA genotypes) was significantly higher in patients with alcohol dependence and alcohol abuse than in healthy controls. The mechanism by which cytokine polymorphisms such as these contribute to alcoholic predisposition remain to be understood but further emphasize the importance of examining cytokine functions in alcohol abuse and alcoholism.

These examples from the alcohol research field are exciting first steps in understanding the relationship of alcohol intake and alcohol-induced tissue damage to levels of circulating cytokines. Much more detailed analysis of many more cytokines is needed to understand the global pattern of cytokine levels in the plasma. While the focus of this review is on the use of cytokines in diagnostic biomarkers, these findings will also add mechanistic insight into the effects of alcohol on a variety of organ systems.

With the promise of cytokines as diagnostic markers there is also the potential that cytokines alone will not provide a sufficiently sensitive and specific test. Addition of other circulating proteins, such as growth factors, to a biomarker panel may reach the required accuracy. Interactions of alcohol with levels of growth factor during pregnancy highlight the potential roles of circulating factors in neurodevelopment. Studies have examined peripheral hepatocyte growth factor (HGF), epidermal growth factor (EGF) and placenta growth factor (PlGF) in pregnant women consuming alcohol (Vuorela et al., 2002). The data indicate that alcohol abuse during pregnancy has a profound effect on circulating EGF and PlGF but not HGF concentrations. However, the data showed no associations between clinical outcome such as birth weight, Apgar score and placental weight and maternal circulating cytokines analyzed during pregnancy. Interestingly, although there were clear differences in the cytokine concentrations between alcohol abusing and alcohol abstinent women, the changes were speculated to be due to altered expression, tissue release or breakdown of these cytokines and thus could not be correlated. Thus, the changes occurring during pregnancy could be primarily due to alcohol or could be part of a cascade of secondary events leading to alcohol-induced placental pathophysiology (Vuorela et al., 2002).

Conclusions

From the above discussion, it is clear that cytokines such as TNF alpha, IL1-alpha, IL1-beta, IL6, IL8, IL12 and MCP-1, can be regulated by alcohol and alcohol-induced tissue damage. As it has become increasingly clear that, in chronic alcoholic patients, the levels of circulating inflammatory cytokines produced by monocytes and macrophages is distorted. Further studies will be needed to determine the course of these changes over the pathological sequelae of alcoholism. The literature available on this aspect is constantly being updated, and a consensus on the pattern of cytokine changes remains to be reached. The complex interactions of these cytokines, such as release of IL-6 in response to TNF alpha remain to be understood (Sheron et al., 1991). Similarly, the role of other factors in cytokine release associated with alcoholism, such as nutrition, age, sex, and method of analysis and co-morbid drug use remains to be examined. However, given the existing literature, there is great promise in using circulating cytokine levels as diagnostics of alcohol abuse and alcoholism. Cytokines, in combination with other circulating proteins, such as the growth factors may provide highly accurate diagnostics of alcohol intake and alcohol-induced tissue damage. These biomarkers could also be measured using existing technology, speeding the translation of new diagnostics. Ultimately, the success or failure of biomarkers of alcohol intake and alcohol-induced tissue damage will depend on the ability of biomarkers to improve patient treatment in a cost effective manner (Kapoor et al., 2009).

Acknowledgments

This work was supported by NIH grant R01AA016613 (KEV). The authors thank Mr. Steven Swavely for his editorial contribution.

Sources of Support: This work was supported by NIH grant R01AA016613 (KEV)

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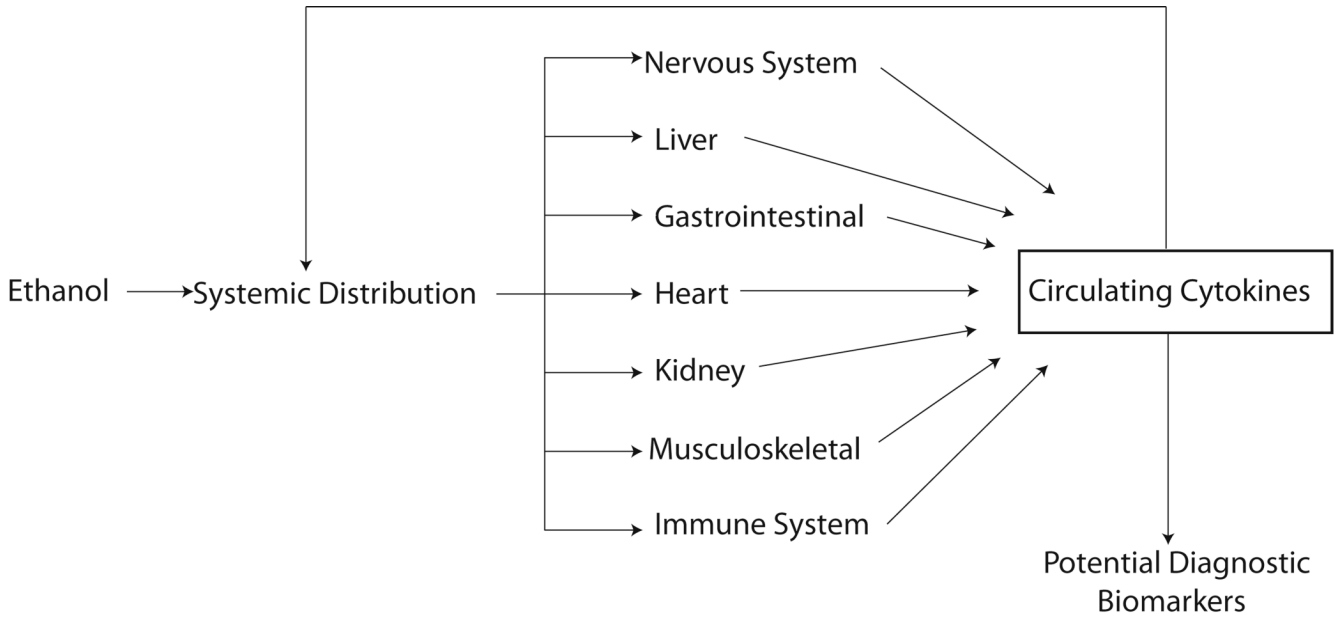

Figure 1.
Alterations of circulating cytokines in response to ethanol originate in a variety of tissues/organs. With increasing amount and duration of ethanol administration, initial changes in cytokine levels may feedback on the body causing secondary changes. Either individually or as panels, circulating cytokines may serve as diagnostic markers of alcohol abuse and alcohol-induced tissue damage.

Table 1

DSM-IV Criteria for Alcohol Abuse and Alcohol Dependence

Alcohol Abuse (1 or more criteria for over 1 year)
• Role Impairment (e.g. failed work or home obligations)
• Hazardous use (e.g. Driving while intoxicated)
• Legal problems related to Alcohol use
• Social or interpersonal problems due to Alcohol
Alcohol Dependence (3 criteria for over 1 year)
• Tolerance (increased drinking to achieve same effect)
• Alcohol Withdrawal signs or symptoms
• Drinking more than intended
• Unsuccessful attempts to cut down on use
• Excessive time related to Alcohol (obtaining, hangover)
• Impaired social or work activities due to Alcohol
• Use despite physical or psychological consequences

Table 2

Summary of potential cytokine changes implicated during human alcoholism

Human alcoholism and associated pathology	Source	Cytokine	Reference
Actively drinking with no chronic liver disease	Plasma	TNF-α↔	Khoruts et el., 1991
		IL1↔	
		IL6↔	
Non-drinking alcoholic with stable cirrhosis		TNF-α↑	
		IL1↑	
		IL6↑	
Acute alcoholic hepatitis		TNF-α↑	
		IL1↑	
		IL6↑	
Alcoholic	Brain	MCP-1↑	He and Crews, 2008
Light/moderate/heavy drinkers with no liver disease	Serum	TNF-α↔	Gonzalez-Quintela et al., 2008
Alcoholics with liver cirrhosis		TNF-α↑	
Alcoholic hepatitis 1990)	Plasma	TNF-α↑	Felver et al., 1990
		IL1-α↑	
		IL1-β↔	
Alcoholic cirrhosis		TNF-α↔	
Alcoholics with no hepatitis		TNF-α↔	
Severe alcoholic hepatitis 1991)	Plasma	IL6↑↑	Sheron et al., 1991
Alcoholic cirrhosis		IL6↑	
Alcoholic without liver disease/ inactive alcoholic cirrhosis/ non-alcoholic chronic liver disease/ chronic liver failure		IL6↔	
Alcoholic hepatitis	Plasma	TNF-α↑↑	Bird et al., 1990
Inactive alcoholic cirrhosis		TNF-α↑	
Alcoholic without liver disease		TNF-α↔	
Alcoholic hepatitis	Serum	IL6↑	Fujimoto et al., 2000
		IL8↑	
		TNF-α(nd)	
Alcoholic hepatitis and cirrhosis		IL6↑	
		IL8↑	
		TNF-α(nd)	
Chronic alcoholic without liver disease	PB monocytes	IL1-β↑	Laso et al., 2007
		IL6↑	
		IL12↑	
		TNF-α↑	

Human alcoholism and associated pathology	Source	Cytokine	Reference
Alcoholic with Liver cirrhosis		IL1-β↔	
		IL6↔	
		IL12↔	
		TNF-α↔	
Chronic alcoholic with liver disease		IL1-β↔	
		IL6↔	
		IL12↔	
		TNF-α↔	
No or mild liver hepatitis/ liver cirrhosis	Plasma	TNF-α↔	Le Moine et al., 1995

Note: nd, not detected, ↔ no change, ↑ slight increase, ↑↑ significant increase