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Critical Evaluation of the Association Between Elevated Mean Corpuscular Volume and Alcohol-Related Traffic Accidents: A Retrospective Study on 6244 Car Crash Cases

Nadia M. Porpiglia, Federica Bortolotti, Romolo M. Dorizzi, Rocco Micciolo, and Franco Tagliaro

Background: Erythrocyte mean corpuscular volume (MCV) has been used for decades as a biomarker of chronic alcohol abuse and in the treatment of alcohol dependence. More recently, it has also been adopted to investigate the fitness of subjects to hold the driving license to prevent traffic accidents. So far, however, the studies on the association of MCV with an increased risk of alcohol-associated car accidents are extremely scarce, if not totally absent. To the best of our knowledge, the present work is the first specifically aimed at studying a plausible association between elevated MCV and crash accidents correlated with alcohol abuse.

Methods: A total of 6,244 drivers involved in traffic accidents underwent mandatory laboratory analyses including blood alcohol concentration (BAC) determination and MCV analysis. BAC and MCV determinations were performed by headspace gas chromatography and complete blood count, respectively.

Results: The chi-square test evaluating the proportions of subjects with elevated MCVs (>95 fl) yielded a highly significant result ($\chi^2 = 68.0$; $p < 0.001$) in the blood samples where the BAC was above the legal limit (i.e., >0.5 g/l). However, when considering only drivers showing BACs in the range of 0.51 to 1.5 g/l, the frequencies of elevated MCV values are fairly comparable ($\chi^2 = 0.062$, $p = 0.80$). In contrast, limiting the evaluation to BACs > 1.5 g/l, the frequency of elevated MCVs raised to 19.1% ($\chi^2 = 58.9$, p value < 0.001 vs. the group with BAC within the legal limits).

Conclusions: The present observations show that MCV increases are typically associated with drivers involved in accidents only if driving under severe alcohol intoxication, leading to a preliminary conclusion that, in the context of the certification of the fitness to the driving license, MCV fails to reveal individuals at risk who tend to drive in a condition of low-to-moderate alcohol intoxication.

Key Words: Mean Corpuscular Volume, Blood Alcohol Concentration, Alcohol-Related Traffic Accident, Driving License, Drunk Driving.

SINCE THE 1960S, with the fundamental "Grand Rapids Study" (a case–control study carried out in Michigan, USA, in 1964) (Borkenstein et al., 1974; Peden et al., 2004; Walker, 2000), the concern on the alcohol-related traffic accidents and their consequences on the individuals' health and on the society has led to actions aimed at contrasting driving under the influence (DUI) of alcohol. In this context, legal blood alcohol limits have been set in almost every country and this policy is considered a milestone in the efforts to reduce traffic accident–related deaths and disability.

If there is a widespread evidence-based consensus on the correlation between the impairment of the driving ability and the blood alcohol concentration (BAC), much weaker evidence can be found in the current literature on the correlation between the chronic abuse of alcohol and an increased risk of traffic accidents. It should be stressed that this point is of the highest importance, particularly when individuals, whose driving license has been withdrawn for "drunk driving," apply for the regranting of their license, pretending to have ceased the abuse of alcohol. In most countries, this delicate matter is under the responsibility of specialized medical committees, who have the task to verify, in any individual, the reality of the alleged abstinence from alcohol. In addition, the medical committee should exclude any reasonable risk of relapse to alcohol abuse and consequently any risk of "driving while intoxicated" in the near future.

Traditionally, the approach to this difficult problem was merely based on medical visits, patient interviews, and few

Department of Diagnostics and Public Health, Unit of Forensic Medicine, University of Verona, Verona, Italy; U.O. Patologia Clinica, (RMD), Laboratorio Unico della Romagna, Cesena, Italy; Department of Psychology and Cognitive Sciences, (RM), University of Trento, Trento, Italy; and Institute Translational Medicine and Biotechnology, (FT), Sechenov First Moscow State Medical University, Moscow, Russia.

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Reprint requests: Federica Bortolotti, MD, PhD, Unit of Forensic Medicine, Department of Diagnostics and Public Health, University of Verona, Piazzale L. A. Scuro 10, 3734, Verona, Italy; Tel.: +39-0458124618; Fax: +39-0458027623; E-mail: federica.bortolotti@univr.it

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clinical laboratory data, including liver enzymes and mean corpuscular volume (MCV).

More recently, in most European countries, new biomarkers have been adopted to objectively provide or exclude evidence of chronic alcohol abuse of the subject (Bortolotti and Tagliaro, 2011; Iffland, 1996; Mercier-Guyon, 1996; Musshoff and Daldrup, 1998). Among these biomarkers, carbohydrate-deficient transferrin (CDT) has found wide application (Arndt, 2001; Crivellente et al., 2000; Helander et al., 2003; Lanz et al., 2004; Legros et al., 2003; Schellenberg et al., 2007), with the support of a few studies including retrospective (Appenzeller et al., 2005b; Bianchi et al., 2010; Bortolotti et al., 2007; Brinkmann et al., 2002; Gjerde and Mørland, 1987; Iffland and Grassnack, 1995; Iffland et al., 1994; Jaster and Wegener, 1993; Kristenson and Jeppsson, 1998) and prospective (Appenzeller et al., 2005a; Maenhout et al., 2004; Marques et al., 2009; Portman et al., 2010) researches. Also, it has recently been reported that elevated CDT concentrations are associated with an increased risk of DUI and, more importantly, with an increased risk of alcohol-related traffic injuries (Bortolotti et al., 2015).

Notwithstanding distinct advantages of using CDT as a biomarker in driving license regranting programs, the use of this diagnostic parameter shows limitations related to analytical complexity and costs, particularly in countries where Public Health budgets are limited.

In this context, MCV is a standard hematological parameter currently measured at low costs in any hospital laboratory by using sound and reliable technologies. In addition to a number of clinical conditions characterized by macrocytosis, elevated MCV is a typical morphological anomaly caused by excessive consumption of alcohol. As a biomarker of chronic alcohol abuse, MCV has been traditionally used for decades in clinical medicine and in the treatment of alcohol dependence showing a sensitivity ranging from 40 to 80% (Neumann and Spies, 2003) with reported substantial differences between females (86%) and males (63%) (Morgan et al., 1981; Sillanaukee et al., 1998). Other reports pointed out an age-dependent sensitivity to detect alcohol abuse (Caputo et al., 2012; Conigrave et al., 2003). On the other hand, the use of MCV as an alcohol biomarker shows a high diagnostic specificity (over 90%) (Meerkerk et al., 1999; van Pelt, 1997), even if other medical conditions may increase MCV and may reduce specificity in nonhealthy individuals (e.g., hematological diseases, vitamin B_{12} and folic acid deficiency, liver diseases, hypothyroidism, myelodysplastic syndromes) (Niemelä and Parkkila, 2004). Little is known about the cause of alcohol-related macrocytosis; however, ethanol (EtOH) is thought to have a direct toxic effect on hemopoiesis since it can permeate the cell membranes and alter the structural order of lipids, possibly affecting erythrocyte stability (Niemelä, 2007).

On these grounds, MCV is widely used as a reliable biomarker of chronic alcohol abuse in the process of certification of the fitness to the driving license and to hold safety-sensitive jobs. However, to the best of our knowledge, no specific studies on the association of increased MCV with the risk of accidents have, so far, been performed.

The present work has been aimed at studying a conceivable, but never adequately proved, association between elevated MCV and alcohol-related traffic accidents, providing important new information particularly in the field of the certification of the physical fitness for holding the driving license, where increased MCV values often lead to a negative outcome of the procedure.

MATERIALS AND METHODS

The present study was carried out on cases collected in the Italian region named Romagna, and particularly from the hospitals of Ravenna, Rimini, Forlì, and Cesena in 2017.

The study included 6,244 subjects (age: 18 to 80, mean: 42.4, standard deviation [SD]: 15.5, median: 42; sex: 4,115 males, 2,129 females) admitted to hospital after a major traffic accident with injuries of different severity, but all still alive. As drivers of the vehicles involved in the crashes, at the time of the admission to the emergency department they were by law tested for BAC. The same subjects were also subjected to hematological tests for clinical purposes, including MCV.

On the basis of BAC values, the subjects were classified into 3 groups:

- group A: BAC > 0.5 g/l
- group B: 0.5 < BAC < 1.5 g/l
- group C: BAC > 1.5 g/l

[Correction added 20 May 2019. Group B incorrectly listed the values as greater than 1.5 g/l.]

taking into consideration that 0.5 g/l corresponds to the Italian legal limit to drive, while 1.5 g/l is the value above which the subject incurs the most severe penalties.

On the basis of MCV values, the subjects were divided into 2 categories:

- category 1: MCV < 95 fl
- category 2: MCV > 95 fl

The choice of using a value of 95 fl to distinguish between non–alcohol abusers and alcohol abusers depended on the cutoff used in the laboratories where MCV determination was performed, taking also into consideration that this value is within the MCV reference intervals reported in the literature (Dorizzi et al., 2000; Pekelharing et al., 2010).

BAC measurement was performed with a headspace GC technique using a Young Lin 6100 fully optimized headspace Gas Chromatography (GC) analyzer with advanced pneumatic control and flame ionization detector (Young Lin Instrument Co., Ltd., Anyang, Korea). Whole blood was mixed 1:4 with the internal standard tert-butyl alcohol (Carlo Erba Reagents, Cornaredo, Italy) at 0.0975 g/l (1 mmol/l). A total volume of 1,250 μl of sample was injected in the system. The temperature of the syringe was 80°C, and the isotherm separation was carried out at 40°C. An internal quality standard provided by ACQ Science GmbH (Rottenburg am Neckar, Germany) with an EtOH concentration of 0.2 g/l (4.4 mmol/l) was measured every 5 real samples. The lower limit of detection (LOD) of this technique was 0.01 g/l (0.22 mmol/l).

Complete blood count was carried out using a model XE-2100 analyzer (Sysmex, Kobe, Japan) following the manufacturer's recommendations, and the results were directly transmitted to the laboratory information system (Dedalus, Florence, Italy). The health-related intervals (HRI) were calculated using the indirect method proposed by Kairisto and Poola (1995). The HRI were

calculated using GraphROC for Windows, a software program for clinical test evaluation, kindly provided by Dr. Kairisto. The reference intervals were calculated using the C28-A3c CLSI standard (Clinical and Laboratory Standards Institute, Katayev et al. 2010) by the software MedCalc v.18.9 (Odense, Belgium).

Owing to the mandatory feature of the BAC determination, no specific ethical approval was required for blood collection and data use. All data were anonymized before use.

The statistical analysis was carried out using nonparametric methods and particularly the chi-square test and the calculation of odds ratio (OR). OR is a numerical expression which attempts to quantify the strength of the association between 2 parameters, A and B [OR expresses the ratio of the odds of A in the presence of B to the odds of A without the presence of B]. If the OR is greater than 1, then A is considered to be associated with B in the sense that, compared to the absence of B, the presence of B raises the odds of A.

RESULTS

The studied population, classified as detailed in the "Materials and Methods" section, shows the following descriptive statistical figures:

- group A (BAC > 0.5 g/l, n = 2,718): average MCV = 87.81 fl (SD = 7.17)
- group B (0.5 < BAC < 1.5 g/l, n = 905): average MCV = 87.85 fl (SD = 7.06)
- group C (BAC > 1.5 g/l, n = 2,621): average MCV = 89.19 fl (SD = 7.07).

[Correction added 20 May 2019. Group B incorrectly listed the values as greater than 1.5 g/l.]

The "chi-square" significance test was first applied to check the "null hypothesis" of absence of difference in the proportions of elevated MCV between the studied groups. Furthermore, the degree of association between the occurrence of elevated MCV values and alcohol-related accidents was evaluated by calculating the odds ratio.

By using the above-mentioned subdivision, the studied cases were classified as in Table 1.

The proportions of subjects with elevated MCVs were 11.6, 11.9, and 19.1% in groups A, B, and C, respectively. The chi-square test yielded a highly significant result (χ^2 = 68.0; $p < 0.001$). At a closer look, however, it is patent that limiting the comparison to group B versus group A, the frequencies of elevated MCV values are fairly comparable, with no significant difference in the proportions (χ^2 = 0.062, p = 0.80). On the other hand, when considering group C (BAC > 1.5 g/l) for the comparison with group A, the frequency of elevated MCV values raised to 19.1% (χ^2 = 58.9; $p < 0.001$).

Table 1. Classification of Groups A, B, and C Versus Categories 1 and 2

	MCV < 95 fl	MCV > 95 fl
Group A	2,404	314
Group B	797	108
Group C	2,119	502

[Correction made 13 May, 2019. Column 1 incorrectly listed the values as greater than 95 fl.]

DISCUSSION

These observations clearly show that MCV is typically increased only in drivers involved in an accident under severe alcohol intoxication. In fact, MCV does not increase in subjects driving with BAC levels above the legal limit but below those associated with the heavier symptoms, that is, >0.5 but ≤1.5 g/l. These findings may lead to a preliminary conclusion that, in the context of the certification of the fitness to the driving license, MCV fails to reveal an important class of individuals at risk, that is, those who tend to drive in a condition of low-to-moderate alcohol intoxication.

When evaluating the strength of the association between the 2 considered factors (BAC at the time of the accident and MCV), an odds ratio of 1.81 was found when comparing the frequencies of elevated MCV values in group C versus group A (the odds were 502/2,119 in group C and 314/2,404 in group A). Despite the significant result associated with this odds ratio (which can be considered significantly different from the "null" value 1, meaning no association at all), in our opinion the strength of the association can be considered weak, limiting the practical usefulness of MCV even for the identification of the conditions of the highest risk for the traffic safety.

The present retrospective study, for logistic reasons, has not included other biomarkers of chronic alcohol abuse, such as CDT, which in recent papers have shown an impressively high association with the risk of alcohol-related traffic accidents (Bortolotti et al., 2015). Further studies will be needed to have a deeper insight in this intricate topic.

CONCLUSIONS

In the last decades, important efforts have been undertaken worldwide with the intent of increasing the traffic safety, particularly by reducing the alcohol-associated traffic accidents. Among these efforts, the adoption of strict procedures for the verification of the fitness to hold the driving license, hindering alcohol abusers from driving vehicles, is one of the most relevant. Unfortunately, neither clinical signs nor biomarkers have, so far, been clearly identified which can undoubtedly indicate that an individual shows an increased risk of being involved in a traffic crash because of their drinking habits. In this context, MCV stands out among the biomarkers of chronic alcohol abuse most traditionally used in the process of certification of the fitness to the driving license. Nevertheless, to the best of our knowledge, notwithstanding its adoption in many countries, the literature is lacking for specific studies on the association between increased MCV values and the occurrence of road accidents. The present study has been aimed at verifying such hypothesis.

A general evaluation of the results of the present retrospective study shows a neat statistical difference in the frequency of elevated MCV values in the blood samples of the drivers in which at the time of the crash the BAC was above the legal limit in comparison with the samples with BAC values within the legal limits. However, when considering only drivers showing BACs between 0.51 and 1.5 g/l, no statistical

difference was found in the frequency of elevated MCV values. On the contrary, limiting the evaluation to BACs > 1.5 g/l, the frequency of elevated MCV values raised to 19.1%. Unfortunately, the degree of the association, quantified by means of the odds ratio, despite the statistical significance, can be considered low for practical purposes.

This clearly leads to a preliminary conclusion that MCV is typically increased only in drivers involved in an accident under severe but not in low-to-moderate alcohol intoxication conditions. Hence, in the context of the certification of the fitness to the driving license, MCV fails to reveal an important class of individuals at risk.

In particular, the low odds ratios of the associations, even when statistically significant, reduce the predictivity of this parameter and consequently its practical usefulness for certifying the fitness of individuals to hold the driving license.

REFERENCES

Appenzeller BMR, Schneider S, Maul A, Wennig R (2005a) Relationship between blood alcohol concentration and carbohydrate-deficient transferrin among drivers. Drug Alcohol Depend 79:261–265.

Appenzeller BMR, Schneider S, Yegles M, Maul A, Wennig R (2005b) Drugs and chronic alcohol abuse in drivers. Forensic Sci Int 155:83–90.

Arndt T (2001) Carbohydrate-deficient transferrin as a marker of chronic alcohol abuse: a critical review of preanalysis, analysis, and interpretation. Clin Chem 47:13–27.

Bianchi V, Ivaldi A, Raspagni A, Arfini C, Vidali M (2010) Use of carbohydrate-deficient transferrin (CDT) and a combination of GGT and CDT (GGT–CDT) to assess heavy alcohol consumption in traffic medicine. Alcohol Alcohol 45:247–251.

Borkenstein RF, Crowther RF, Shumate RP (1974) The role of the drinking driver in traffic accidents (the Grand Rapids study). Blutalkohol 11:1–131.

Bortolotti F, Micciolo R, Canal L, Tagliaro F (2015) First objective association between elevated carbohydrate-deficient transferrin concentrations and alcohol-related traffic accidents. Alcohol Clin Exp Res 39:2108–2114.

Bortolotti F, Tagliaro F (2011) Biomarkers for the identification of alcohol use/abuse: a critical review. Forensic Sci Rev 23:56–72.

Bortolotti F, Trettene M, Gottardo R, Bernini M, Ricossa MC, Tagliaro F (2007) Carbohydrate-deficient transferrin (CDT): a reliable indicator of the risk of driving under the influence of alcohol when determined by capillary electrophoresis. Forensic Sci Int 170:175–178.

Brinkmann B, Beike J, Köhler H, Heinecke A, Bajanowski T (2002) Incidence of alcohol dependence among drunken drivers. Drug Alcohol Depend 66:7–10.

Caputo F, Vignoli T, Leggio L, Addolorato G, Zoli G, Bernardi M (2012) Alcohol use disorders in the elderly: a brief overview from epidemiology to treatment options. Exp Gerontol 47:411–416.

Conigrave KM, Davies P, Haber P, Whitfield JB (2003) Traditional markers of excessive alcohol use. Addiction 98:31–43.

Crivellente F, Fracasso G, Valentini R, Manetto G, Riviera AP, Tagliaro F (2000) Improved method for carbohydrate-deficient transferrin determination in human serum by capillary zone electrophoresis. J Chromatogr B 739:81–93.

Dorizzi RM, Schinella M, Pupillo A, Endrizzi L (2000) Hematological health-related intervals estimated using an indirect method in order to satisfy the accreditation standards. Accred Qual Assur 5:367–370.

Gjerde H, Mørland J (1987) Concentrations of carbohydrate-deficient transferrin in dialysed plasma from drunken drivers. Alcohol Alcohol 22:271–276.

Helander A, Husa A, Jeppsson JO (2003) Improved HPLC method for carbohydrate-deficient transferrin in serum. Clin Chem 49:1881–1890.

Iffland R (1996) New ways to use biochemical indicators of alcohol abuse to regrant licences in a fairer manner after drunken driving in Germany. Alcohol Alcohol 31:619–620.

Iffland R, Balling MP, Börsch G, Herold C, Kaschade W, Löffler T, Schmidtmann U, Stettner J (1994) Evaluation of an increased blood level of GGT, CDT, methanol, acetone and isopropanol in alcohol intoxicated automobile drivers. Alcoholism indicators instead of medical-psychological examination. Blutalkohol 31:273–314.

Iffland R, Grassnack F (1995) Epidemiologic study of CDT (carbohydrate-deficient transferrin) and other indicators of alcohol problems in high blood alcohol German automobile drivers. Blutalkohol 32:26–41.

Jaster R, Wegener R (1993) Diagnosis of chronic alcohol abuse in intoxicated drivers: carbohydrate-deficient transferrin (CDT) in combination with other parameters. Blutalkohol 30:257–265.

Katayev A, Balciza C, David W, Seccombe DW (2010) Establishing reference intervals for clinical laboratory test results. Is there a better way? Am J Clin Pathol 133:180–186.

Kairisto V, Poola A (1995) Software for illustrative presentation of basic clinical characteristics of laboratory tests-GraphROC for Windows. Scand J Clin Lab Invest Suppl 222:43–60.

Kristenson H, Jeppsson JO (1998) Drunken driver examinations. CD-transferrin is a valuable marker of alcohol consumption. Läkartidningen 95:1425–1426; 1429–1430.

Lanz C, Kuhn M, Deiss V, Thormann W (2004) Improved capillary electrophoresis method for the determination of carbohydrate-deficient transferrin in patient sera. Electrophoresis 25:2309–2318.

Legros FJ, Nuyens V, Baudoux M, Zouaoui Boudjeltia K, Ruelle JL, Colicis J, Cantraine F, Henry JP (2003) Use of capillary zone electrophoresis for differentiating excessive from moderate alcohol consumption. Clin Chem 49:440–449.

Maenhout TM, Poll A, Vermassen T, De Buyzere ML, Delanghe JR (2004) Usefulness of indirect alcohol biomarkers for predicting recidivism of drunk-driving among previously convicted drunk-driving offenders: results from the Recidivism Of Alcohol-impaired Driving (ROAD) study. Addiction 109:71–78.

Marques P, Tippetts S, Allen J, Javors M, Alling C, Yegles M, Pragst F, Wurst F (2009) Estimating driver risk using alcohol biomarkers, interlock blood alcohol concentration tests and psychometric assessments: initial descriptives. Addiction 105:226–239.

Meerkerk GJ, Njoo KH, Bongers IM, Trienekens P, van Oers JA (1999) Comparing the diagnostic accuracy of carbohydrate-deficient transferrin, γ-glutamyltransferase, and mean cell volume in a general practice population. Alcohol Clin Exp Res 23:1052–1059.

Mercier-Guyon C (1996) Evaluation of alcohol consumption level in drivers when regranting licences after driving while intoxicated in France. Alcohol Alcohol 31:617–618.

Morgan MY, Camilo ME, Luck W, Sherlock S, Hoffbrand AV (1981) Macrocytosis in alcohol-related liver disease: its value for screening. Clin Lab Haematol 3:35–44.

Musshoff F, Daldrup T (1998) Determination of biological markers for alcohol abuse. J Chromatogr B Biomed Sci Appl 713:245–264.

Neumann T, Spies C (2003) Use of biomarkers for alcohol use disorders in clinical practice. Addiction 98(Suppl. 2):81–91.

Niemelä O (2007) Biomarkers in alcoholism. Clin Chim Acta 377:39–49.

Niemelä O, Parkkila S (2004) Alcoholic macrocytosis—is there a role for acetaldehyde and adducts? Addict Biol 9:3–10.

Peden M, Scurfield R, Sleet D, Mohan D, Hyder AA, Jarawan E, Mathers C (2004) World Report on Road Traffic Injury Prevention. World Health Organisation, Geneva, Switzerland.

Pekelharing JM, Hauss O, de Jonge R, Lokhoff J, Sodikromo J, Spaans M, Brouwer R, de Lathouder S, Hinzmann R (2010) Haematology reference intervals for established and novel parameters in healthy adults Diagnostic. Perspectives 1:1–11.

Portman M, Penttilä A, Haukka J, Eriksson P, Alho H, Kuoppasalmi K (2010) Predicting DUI recidivism of male drunken driving: a prospective study of the impact of alcohol markers and previous drunken driving. Drug Alcohol Depend 106:186–192.

Schellenberg F, Girre C, Nalpas B, Plat A, Tome A, Pagès JC (2007) Analytical evaluation of a new capillary electrophoresis method for carbohydrate-deficient transferrin measurement. Clin Chim Acta 382:48–53.

Sillanaukee P, Aalto M, Seppä K (1998) Carbohydrate-deficient transferrin and conventional alcohol markers as indicators for brief intervention among heavy drinkers in primary health care. Alcohol Clin Exp Res 22:892–896.

van Pelt J (1997) Carbohydrate-deficient transferrin: a new biochemical marker for chronic excessive alcohol consumption. Ned Tijdschr Geneeskd 141:773–777.

Walker PF (2000) Epidemiology of alcohol-related accidents and the grand rapids study. Forensic Sci Rev 12:107–118.