DISSERTATIONES
MEDICINAE
UNIVERSITATIS
TARTUENSIS
327

JANA TUUSOV

Deaths caused by alcohol, psychotropic and other substances in Estonia: evidence based on forensic autopsies

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
327

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
327

JANA TUUSOV

Deaths caused by alcohol, psychotropic and other substances in Estonia: evidence based on forensic autopsies

UNIVERSITY OF TARTU
Press
1632

Department of Pathological Anatomy and Forensic Medicine, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia

Dissertation accepted for the commencement of the degree of Doctor of Philosophy in Medicine on March 16th, 2022 by the Council of the Faculty of Medicine, University of Tartu, Estonia.

Supervisors: Professor Marika Väli, MD, PhD
Department of Pathological Anatomy and Forensic Medicine
Institute of Biomedicine and Translational Medicine
University of Tartu, Estonia

Associate professor Kersti Pärna, MD, MPH, PhD
Department of Public Health
Institute of Family Medicine and Public Health
University of Tartu, Estonia

Associate professor Katrin Lang, MD, MPH, PhD
Department of Epidemiology and Biostatistics
Institute of Family Medicine and Public Health
University of Tartu, Estonia

Reviewers: Professor Andres Arend, MD, PhD
Department of Anatomy
Institute of Biomedicine and Translational Medicine
University of Tartu, Estonia

Associate professor Kadri Suija, MD, PhD
Department of Family Medicine
Institute of Family Medicine and Public Health
University of Tartu, Estonia

Opponent: Professor Philippe Lunetta MD, PhD
Forensic Medicine, Institute of Biomedicine
University of Turku, Finland

Commencement: June 14th, 2022

Publication of this dissertation is granted by the University of Tartu.

This research was supported by the Institutional Research Funding from the Estonian Research Council and by the European Union through the European Regional Development Fund.

ISSN 1024-395X
ISBN 978-9949-03-888-6 (print)
ISBN 978-9949-03-889-3 (pdf)

University of Tartu Press
www.tyk.ee

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following publications referred to in the text by Roman numerals (I–III):

I. Ringmets I, Tuusov J, Lang K, Väli M, Pärna K, Tõnisson M, Helander, A, McKee M, Leon DA. Alcohol and premature death in Estonian men: a study of forensic autopsies using novel biomarkers and proxy informants. BMC Public Health. 2012;12:146.

II. Tuusov J, Lang K, Väli M, Pärna K, Tõnisson M, Ringmets I, McKee M, Helander A, Leon DA. Prevalence of alcohol-related pathologies at autopsy: Estonian forensic study of alcohol and premature death. Addiction. 2014;109:2018–2026.

III. Tuusov J, Vals K, Tõnisson M, Riikoja A, Denissov G, Väli M. Fatal poisoning in Estonia 2000–2009. Trends in illegal drug-related deaths. Journal of Forensic and Legal Medicine 2013;20:51–56.

Contribution of Jana Tuusov (JT) to the original publications:

Paper I: JT participated in the study design, in the collection and interpretation of the data, in the writing of the manuscript and critically revised the manuscript.

Paper II: JT participated in the study design, in the collection and interpretation of the data, and wrote the first draft of the manuscript.

Paper III: JT participated in the study design, in the interpretation of the data, and wrote the first draft of the manuscript.

ABBREVIATIONS

ALT	alanine aminotransferase
AST	aspartate aminotransferase
BAC	blood alcohol concentration
CDT	carbohydrate-deficient transferrin
CO	carbon monoxide
COHb	carboxyhemoglobin
EDTA	ethylenediaminetetraacetic acid
EFSI	Estonian Forensic Science Institute
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
EtG	ethyl glucuronide
EtS	ethyl sulphate
EU	European Union
GGT	gamma-glutamyl transferase
GHB	gamma hydroxybutyrate
ICD-10	10^{th} revision of International Classification of Diseases
MCV	mean corpuscular volume
MDMA	3, 4-methylenedioxymethamphetamine
PEth	phosphatidylethanol
RHK-10	Rahvusvaheline Haiguste Klassifikatsioon, 10. versioon
WHO	World Health Organization

1. INTRODUCTION

In Estonia, life expectancy has increased since 1994. In 2019, it was 74.4 for men and 82.8 for women (Statistics Estonia, 2021). Life expectancy in Estonia is still lower than in European Union (EU), whereas gender difference is bigger (World Health Organization, 2021). Gender gap is due, largely, to excess deaths among men of working age, and often related to alcohol consumption.

Heavy alcohol consumption has been implicated as a major cause of premature mortality (Shield & Rehm, 2015; Rehm et al., 2019), with high levels of hazardous drinking in the population (Jasilionis et al., 2020; Leon et al., 1997; Popova et al., 2007). In Estonia, premature mortality, associated with excessive alcohol consumption, is high, especially in men (Bogstrand et al., 2011; Rahu et al., 2019). Another preventable cause of premature death of men in Estonia is poisoning with psychotropic and other substances.

Alcohol can cause or be implicated in death in many ways, both through acute direct and indirect pathways (Bogstrand et al., 2011), and as a result of chronic effects on organs (Britton & McKee, 2000; Lahti et al., 2011; Leon et al., 2010; Mäkelä, 1998; Persson et al., 2013). The amount and distribution of alcohol- related causes of death depends, among other things, on the coding practice and availability of relevant codes of causes of death.

In forensic medicine, chronic alcohol use and organ damage is determined based on autopsy findings and, if available, deceased person's medical records and preliminary data provided by the police. Yet there are biochemical measures traditionally linked with hazardous drinking: gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), mean cell volume and carbohydrate-deficient transferrin (CDT). Novel markers of detecting recent alcohol intake are ethyl glucuronide (EtG) and ethyl sulphate (EtS) and phosphatidylethanol (PEth) for assessing long time alcohol exposure. Alcohol biomarkers can be used in *post mortem* diagnostics of alcohol use, yet their role needs to be determined.

It is important to find out the role of alcohol in causing damage to human body as well as the magnitude of it affecting population health. One of the ways of doing that is to carry out an autopsy-based study. Because of the complicated nature of such studies there are very few of them, but still, they produce valuable information. Similarly, it is important to assess and improve ways of coding causes of death to reveal the role of alcohol in causing deaths, also as reflected in mortality statistics at population level.

The present study explores deaths caused by alcohol among 25–54-years-old working age men using an in-depth forensic autopsy analysis, explores the coding problems of underlying cause of death in presence of multiple alcohol-related pathologies and describes fatal poisonings caused by alcohol, psychotropic and other substances among general population in Estonia. The study is the first in Estonia to use in-depth forensic autopsy to explore the role of alcohol in premature mortality.

2. REVIEW OF THE LITERATURE

Estonia is the most northern of the three Baltic countries, with high burden of premature mortality, especially among men. Life expectancy is one of the measures to quantify the effect of premature mortality at population level and therefore the following paragraphs are dedicated to this topic.

2.1. Life expectancy in Estonia in the context of European Union

Life expectancy has increased steeply since 1994 in Estonia. While in 1994 life expectancy among men was 60.5 and among women 72.8, in 2019 it was 74.4 and 82.8, respectively (Statistics Estonia, 2021) (Figure 1). At the same time, average life expectancy in EU increased steadily from 72.4 to 77.5 years for men and from 78.1 to 83.5 among women in 1994–2015 (World Health Organization, 2021). Alcohol use is impacting life expectancy and has been highlighted as an important contributory reason for the differences in life expectancy between eastern and western Europe (Danilova et al., 2020; McKee & Shkolnikov, 2001; Rehm et al., 2019). In 2015, the gender difference of life expectancy was 8.8 years in Estonia compared to an average of 6.0 years in the EU. The gender gap is due, largely, to excess deaths among men of working age in Estonia, and often related to alcohol. Alcohol-related mortality accounted for 20–30% of the gender gap in life expectancy in eastern Europe (McCartney et al., 2011; Trias-Llimós & Janssen, 2018).

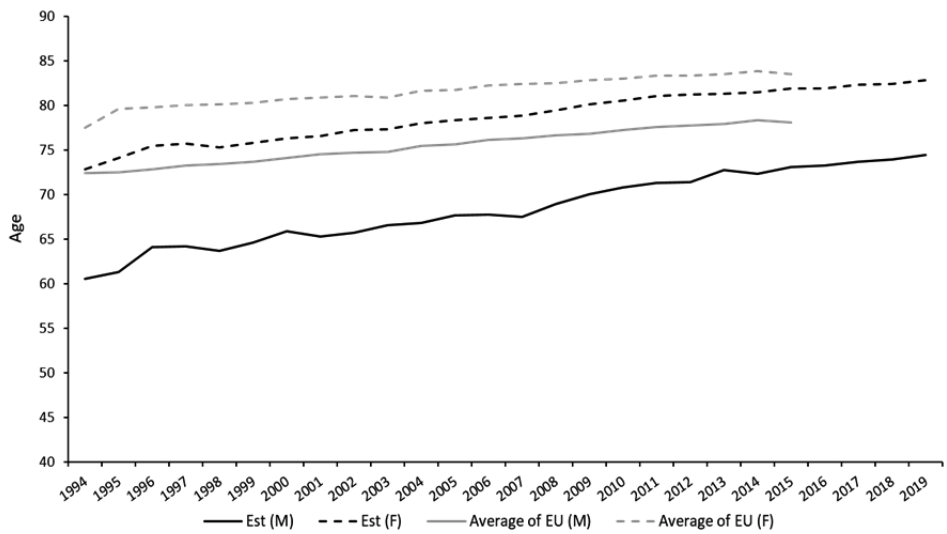


Figure 1. Life expectancy among men (M) and women (W) in Estonia and in European Union (EU) average, 1995–2019 (Statistics Estonia, 2021; World Health Organization, 2021).

2.2. Alcohol consumption in Estonia in the context of European Union

Total domestic alcohol consumption

Total domestic adult (15 years and older) alcohol consumption (measured in pure alcohol in litres per capita) increased from 11.4 to 14.8 in 2004–2007, followed by a decrease to 10.0 in 2018 (10.4 in 2019) (Figure 2) (Estonian Institute of Economic Research, 2020). At the same time average alcohol consumption in EU decreased steadily from 11.2 to 9.9 in 2004–2016 (from 9.4 to 7.9 in WHO European Region) (World Health Organization, 2021).

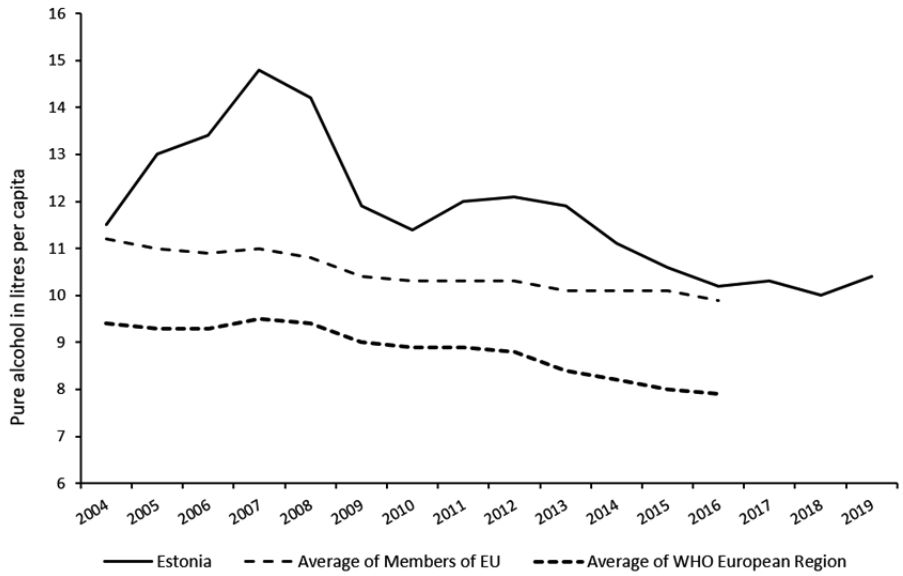


Figure 2. Total alcohol consumption among adults (15-years of age and older) in Estonia, in Member States of EU and WHO European Region (Estonian Institute of Economic Research, 2020; World Health Organization, 2021).

Alcohol consumption by beverage type

In Estonia, consumption of spirits (mainly vodka) among adult population (15 years and older) increased from 3.2 to 6.7 litres of pure alcohol per capita in 2000–2007, and thereafter decreased to 3.8 in 2019. The consumption of beer increased from 4.4 to 5.3 in 2000–2007 and thereafter slightly decreased to 4.1 in 2019. The consumption of light beverages (e.g. cider, long drinks) increased from 0.6 to 1.8 in 2000–2007 and thereafter decreased to 0.6 in 2019. Wine was the one beverage type whose consumption increased most consistently since the mid-2000s to 1.9 in 2019 (Estonian Institute of Economic Research, 2020).

The use of surrogate, industrial and illegal alcohol is often more harmful than common non-beverage alcohol use. Description and use of these types of alcohol is therefore provided in the following section.

Surrogate alcohol consumption

Surrogate alcohol means manufactured substance containing up to 95% ethanol by volume but not officially intended for drinking such as eau de cologne, alcohol-containing medicines, fluids for lighting fires and industrial and technical spirits, including window cleaner) (McKee et al., 2005). Surrogate alcohol drinking is an important component of hazardous drinking. In Izhevsk, Russia, in a sample of men aged 25–54 years reported prevalence of surrogate alcohol drinking was 7% in the middle of the first decade of this century (Tomkins et al., 2007). In Estonia, questionnaire-based study reported the prevalence of surrogate alcohol of 2.3% among 15–84-year-old men in 2006 (Pärna & Leon, 2011). Surrogate alcohol drinking is often associated with alcohol-related harms, including alcohol poisoning and multiple organ damage such as cardiomyopathy, cirrhosis, and ischaemic heart disease (Leon et al., 2007).

Industrial and illegal alcohol consumption

Consumption of industrial spirits containing methanol or illegal alcoholic beverages containing only methanol or mixed with alcohol have caused large outbreaks e.g. in Czech Republic and in Norway (Hovda et al., 2005; Zakharov et al., 2014). In Estonia, illegal alcohol drinking was the cause to death of 68 persons in September 2001 (Paasma et al., 2007). Death is caused by metabolic acidosis from the production of formic and lactic acids, selective toxicity to optical nerve and basal ganglia are well known. In Estonia, illegal alcohol sale decreased from 1.3 litres of pure alcohol per capita (15 years and older) in 2003 to 0.3 in 2019 (Estonian Institute of Economic Research, 2020).

2.3. Alcohol-related harm

In a recent systematic review, 255 reviews and meta-analyses were identified and analysed on alcohol consumption and health outcomes attributable to alcohol use (Rehm et al., 2017). Alcohol use was found to be linked causally to many disease and injury categories, with more than forty 10^{th} revision of ICD-10 categories being fully attributable to alcohol.

Alcohol related harm depends on the concentration and volume of alcohol drunk, as well as pattern of drinking. Particularly spirits, which are more popular in eastern Europe, have stronger health effects (World Health Organization, 2019). While in the beginning of 2000s strong alcoholic beverages were the most consumed in Estonia, since 2010, consumption of beer measured in pure alcohol per capita has been more prevalent in Estonia (Estonian Institute of Economic Research, 2020; Pärna, 2020) eventually hopefully alleviating the burden of alcohol related harm.

The pattern of alcohol use also determines the extent of alcohol-related harm in human body, and some of the patterns are more hazardous or even harmful. In Estonia, in parallel with total alcohol consumption, the proportion of men drinking more than 280 g pure alcohol a week increased in 1994–2006 (Pärna et al., 2010). Compared to Finland, the proportion of heavily drinking men per week was about the same in 1994, but nearly 1.5 times higher in 2006 in Estonia (23.2% and 16.2%, respectively, in 2006). Age-period-cohort analyses in Estonia showed that alcohol consumption among 16–64-years-old men and women increased in 1996–2018 (for men from daily mean of 11.5 g to 15.2 g) (Baburin et al., 2020).

Heavy episodic drinking (defined as 60 or more grams of pure alcohol on at least one single occasion at least once per month), that was more common in the former Soviet Union, is associated with alcohol-related harm (Pomerleau et al., 2008).

Given the limitations of biomarkers in determining the role of alcohol, and the long time for alcohol exposure to take effect, the outcome at organ level is very difficult to be measured in those subjects. Often the most measurable end-points are detected when people die, and for this reason alcohol-related harm is described in relation to mortality and provided in the following section.

2.4. Alcohol-related mortality

Conditions related to alcohol and substance use represent the largest proportion of preventable causes of death. Alcohol accounts for a high proportion of premature mortality in Europe (Rehm et al., 2019; Shield & Rehm, 2015), but heavy drinking has been regarded as an especially important contributor to the high premature mortality rates during the last decades in central and eastern Europe, particularly in countries of the former Soviet Union (Jasilionis et al., 2020; Leon et al., 1997; Popova et al., 2007). In Estonia, premature mortality, associated with excessive alcohol consumption, is high, especially in men (Rahu et al., 2019).

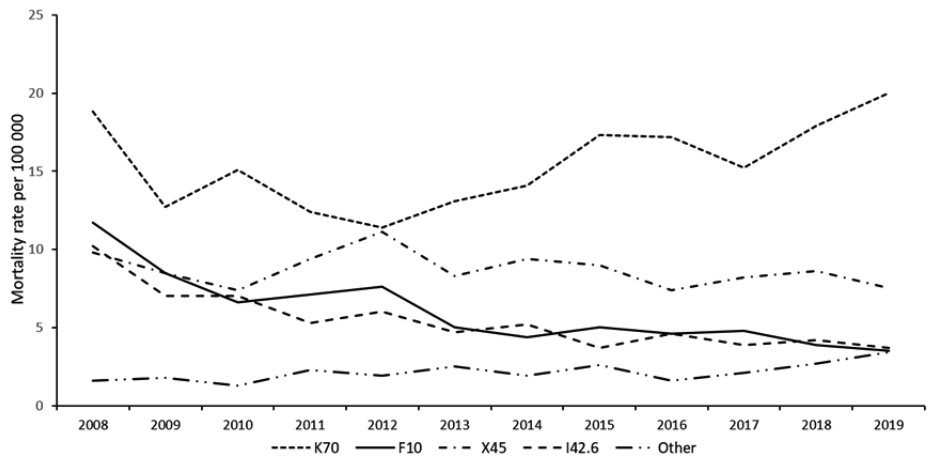
Alcohol can cause or be implicated in death in many ways, both through acute direct (e.g. alcohol poisoning) and indirect (injuries and violence) pathways (Bogstrand et al., 2011), and as a result of chronic effects on organs such as the digestive and cardiovascular systems (Britton and McKee, 2000; Lahti et al., 2011; Leon et al., 2010; Mäkelä, 1998; Persson et al., 2013).

In a study based on data from 17 European countries, deaths from alcoholic psychosis, dependence, and abuse; alcoholic cardiomyopathy; alcoholic liver cirrhosis; and accidental poisoning by alcohol was analysed (Mackenbach et al., 2015). Mortality from all alcohol-related causes together was highest in Hungary, both among men and women, with age-standardized mortality rates of 198.1 (95% CI 195.1–200.9) and 51.4 (95% CI 50.0–52.7) deaths per 100 000 person-years, respectively, mainly due to extremely high mortality from alco-

holic liver cirrhosis. Alcoholic psychosis, dependence, and abuse mortality was highest in Denmark, and alcohol poisoning mortality was highest in Estonia.

Twenty-nine countries within the World Health Organization (WHO) European region were evaluated for trends in alcohol-related deaths (Pruckner et al., 2019). Between 1979 and 2015, age-standardized death rates due to selected alcohol-related causes (cancer of oesophagus and larynx, ICD-10 codes: C15, C32; mental and behavioural disorders due to use of alcohol, F10; chronic liver disease and cirrhosis: K70, K73, K74, K76; and all external causes including traffic accidents, falls, drowning and assault) decreased significantly for both sexes in all assessed countries of the WHO European region, but regional differences were still pronounced. Previously, alcoholic liver cirrhosis mortality rates in Estonia in 1992–2008 have been studied (Pärna & Rahu, 2010), concluding that mortality from alcoholic liver cirrhosis increased steeply over this time period.

As for Estonia, total alcohol related mortality decreased between 2008–2019 (Figure 3): it was 52.0 per 100 000 inhabitants in 2008, and 38.2 in 2019. As for specific alcohol related causes of death, alcoholic liver disease (K70) decreased from 18.8 per 100 000 in 2008 to 11.4 in 2012 and thereafter increased to 20.0 in 2019. Mortality from mental and behavioural disorders due to use of alcohol (F10) decreased steadily from 11.7 to 3.5 in 2008–2019. Mortality from accidental poisoning by exposure to alcohol (X45) was quite stable over two decades being 9.8 in 2008 and 7.5 in 2019. Alcoholic cardiomyopathy (I42.6) mortality decreased steadily from 10.2 in 2008 to 3.7 in 2019. Mortality from other causes (Degeneration of nervous system due to alcohol – G32.1; Alcoholic polyneuropathy – G62.1; Alcoholic gastritis – K29.2; Alcohol-induced chronic pancreatitis – K86.0) was stable in 2008–2016 and thereafter increased to 3.4 in 2019.



Other - (G32.1, G62.1, K29.2, K86.0)

Figure 3. Crude alcohol-related mortality rate per 100 000 inhabitants in Estonia, 2008–2019 (Health Statistics and Health Research Database, 2021).

2.4.1. Alcohol-related mortality caused by acute direct pathways

Alcohol poisoning

Deaths caused by alcohol poisoning – excessive amount of alcohol ingested leading to the depression of the respiratory centre in brain or over aspiration of gastric content to asphyxia are the most frequent causes of poisoning deaths in Estonia. Blood alcohol concentration (BAC) is the main indicator of acute alcohol poisoning. The criterion for lethal alcohol intoxication is BAC value over 3.0 mg/g (Drummer & Odell, 2001). Finnish study of alcohol-positive deaths has shown similar results: the average/median BAC was 3.3/3.3‰ in poisonings where alcohol was the only or main intoxicant and 3.5/3.5‰ where alcohol was the sole intoxicant (Lahti et al., 2011). BAC values in alcohol poisonings can be lower depending on co-existing health conditions and diseases, and other drugs involved. Still, most of the lethal ethanol intoxications are mono-intoxications i.e. involving only one substance (Jones et al., 2011).

2.4.2. Alcohol-related mortality caused by indirect pathways

Alcohol-related injury deaths

Three main groups among injury death are accidents (traffic accidents, fire accidents, drowning, falling, hypothermia), suicides and homicides. Figure 4 shows crude mortality rates from different types of accidents, suicides and homicides in 2005–2019 in Estonia.

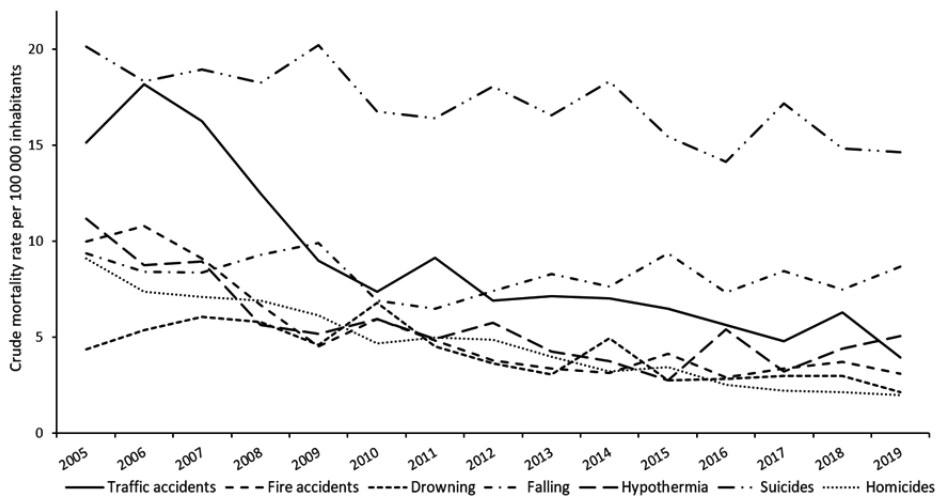


Figure 4. Deaths caused by indirect pathways in 2005–2019 in Estonia (Health Statistics and Health Research Database, 2021).

Accidents

Crude mortality rate for accidents decreased from 81.9 per 100 000 inhabitants in 2005 to 38.1 in 2019 in Estonia.

The crude mortality rate for traffic and fire accidents, drowning and hypothermia decreased in 2005–2019 in Estonia, but mortality from falls fluctuated (Figure 4). In 2005–2019, crude mortality rate for traffic accidents (V01–V99, Y85) decreased from 15.1 to 3.9, for fire accidents (X00–X09) from 10.0 to 3.1, for drowning (W65–W74) from 4.4 to 2.1, for fallings (W00–W19) fluctuated between 9.4 and 8.7 and for hypothermia (X31) decreased from 11.2 to 5.1 per 100 000 inhabitants in Estonia.

Table 1 shows the proportions of fatalities by alcohol intoxication, which are rather remarkable, especially for some of the causes of death. The proportion (%) of fatalities with high (>2.5mg/g) alcohol intoxication varied between 4.0% and 20.7% for traffic accidents, 16.4% and 41.7% for fire accidents, 11.1% and 25.8% for drownings, 5.8% and 14.3% for fallings and between 2.7% and 12.9% for hypothermia in 2014–2019 in Estonia (Estonian Institute of Economic Research, 2020).

Suicides

Crude mortality rate for suicides (X60–X84, Y87.0) decreased from 20.2 per 100 000 inhabitants in 2005 to 14.6 in 2019 in Estonia (Figure 4). Table 1 shows that about one third of persons committed suicide had alcohol intoxication (at least 0.2 mg/g) in 2014–2019 in Estonia. Among these, the proportion of persons with high alcohol intoxication (>2.5 mg/g) varied from 4.1% to 11.0% over these years.

The association between alcohol use and suicide has been proven in several studies (Akechi et al., 2006). A systematic review of literature (Kõlves et al., 2020) concluded that alcohol policies for reduction of alcohol-related harm were in majority associated with reduced suicides across Western and Eastern Europe, as well as the US.

Homicides

Crude mortality rate for homicides (X85–Y09, Y87.1) decreased from 9.1 per 100 000 inhabitants in 2005 to 2.0 in 2019 in Estonia (Figure 4).

Table 1 shows that more than half of homicide victims had alcohol intoxication (at least 0.2 mg/g) in 2014–2019 in Estonia. Among these, the proportion of persons with high alcohol intoxication (>2.5 mg/g) varied from 28.6% to 37.2% over these years.

Table 1. Proportion (%) of fatalities by alcohol intoxication* among external causes of death caused by indirect pathway, 2014–2019 (Estonian Institute of Economic Research, 2020)

Fatalities by alcohol intoxication	2014	2015	2016	2017	2018	2019
Traffic accidents (drivers, pedestrians, cyclists)						
High	14.3	19.3	20.7	4.0	11.1	5.3
Moderate	18.2	21.0	13.8	10.0	14.3	17.5
Low	5.2	1.6	6.9	4.0	6.3	7.0
Sober	49.3	46.8	46.6	62.0	49.2	49.1
Condition not determined	13.0	11.3	12.1	20.0	19.0	21.1
Fire accidents						
High	34.6	30.9	24.4	24.5	16.4	41.7
Moderate	18.4	18.2	21.9	20.4	18.0	4.2
Low	14.3	5.4	12.2	10.2	18.0	4.2
Sober	24.5	27.3	29.3	28.6	37.7	33.3
Condition not determined	8.2	18.2	12.2	16.3	9.8	16.7
Drowning						
High	25.8	11.1	17.5	19.5	13.6	18.4
Moderate	19.4	16.7	17.5	19.5	25.0	31.6
Low	11.3	13.9	5.0	4.9	6.8	10.5
Sober	40.3	52.8	60.0	51.2	43.2	34.2
Condition not determined	3.2	5.5	0.0	4.9	11.4	5.3
Falling						
High	14.3	9.1	5.8	10.3	10.8	10.3
Moderate	13.0	6.1	8.1	11.5	9.5	5.9
Low	6.5	7.1	3.5	5.1	12.2	16.2
Sober	24.7	33.3	34.9	30.8	21.6	36.8
Condition not determined	41.5	44.4	47.7	42.3	45.9	30.9
Hypothermia						
High	6.4	2.7	12.9	9.8	9.3	6.2
Moderate	27.7	24.3	25.7	14.6	22.2	16.9
Low	17.0	18.9	17.1	24.4	13.0	21.5
Sober	38.3	40.6	40.0	41.4	51.9	49.2
Condition not determined	10.6	13.5	4.3	9.8	3.7	6.2
Suicide						
High	6.9	5.5	11.0	6.4	4.1	4.7
Moderate	17.7	17.7	9.2	16.7	14.2	12.6
Low	12.1	8.8	11.7	16.7	10.7	15.3
Sober	54.6	63.0	60.7	56.0	66.5	64.7
Condition not determined	8.7	5.0	7.4	4.2	4.6	2.6
Homicide						
High	30.8	37.2	28.6	35.5	37.0	34.6
Moderate	17.9	16.3	11.4	22.6	14.8	11.5
Low	7.7	11.6	17.1	9.7	0.0	0.0
Sober	35.9	16.3	28.6	22.6	25.9	19.2
Condition not determined	7.7	18.6	14.3	9.7	22.2	34.6

*Alcohol intoxication (high: more than 2.5 mg/g; moderate: 1.5–2.5 mg/g; low: 0.2–1.5 mg/g)

2.4.3. Alcohol-related mortality caused by chronic conditions

In Estonia the main chronic conditions contributing to alcohol-related mortality are alcoholic liver disease, pancreatitis, and cardiomyopathy. Thus, morphological alterations of liver, heart, pancreas, but also lung and gut (intestine) are common findings in alcohol use. Yet these conditions are very hard to detect as practice of coding them as underlying causes of death is not standardised and thus statistics about these pathologies are distorted. If coded as accompanying diseases of death, they are not reflected in routine mortality statistics and only special autopsy studies can shed light on the magnitude of the problem.

2.5. Alcohol-related organ damage

2.5.1. Liver damage

Chronic alcohol use results in alcoholic liver disease, which is one of the most important causes of deaths caused by alcohol. Review and meta-analyses of several cohort and case control studies showed that consuming five or more drinks daily increased risk for liver cirrhosis both in men and women, and the risk for women was higher consistently even in lower amounts of alcohol intake (Roerecke et al., 2019). Alcoholic liver disease includes hepatic steatosis, alcoholic hepatitis, liver fibrosis and cirrhosis, and finally, hepatocellular carcinoma may develop.

The severity and progression of alcoholic liver disease varies between individuals correlating to the duration and amounts of alcohol intake (Chacko & Reinus, 2016). This is also influenced by co-existing conditions e.g. viral hepatitis and obesity (strongly associated with non-alcoholic fatty liver disease) (Zakhari, 2013). Although alcoholic steatosis is reversible with abstinence, continuous consumption of alcohol of over 30 (40) g per day, related to development of fibrosis and cirrhosis, have adverse effect on the outcome of most liver diseases (Hagström, 2017; Teli et al., 1995).

More than 90% of individuals consuming large amounts of alcohol develop steatosis. This is one of the earliest responses of the liver to alcohol abuse and is due to an accumulation of lipid droplets in hepatocytes (Philips et al., 2019; Schulze & Ding, 2019). Hepatocytes are injured by alcohol and its metabolites by various mechanisms leading to oxidative stress, endoplasmatic reticulum and mitochondrial stress. Alcohol causes, directly or indirectly, a rise in the activity of lipogenic pathways and decrease in mitochondrial ß-oxidation – catabolic pathway for degradation of stored fatty acids. Furthermore, reactive oxygen species due to CYP2E1 activity can lead to apoptosis (Schulze & Ding, 2019). Alcoholic hepatitis, characterised by liver tissue infiltration with neutrophils and cellular injury, is believed to develop in 10–30% of alcohol abusers (Gao & Bataller, 2011). Histological features of alcoholic hepatitis, in addition to neutrophilic reaction, are swelling/ballooning and necrosis of hepatocytes, presence of Mallory bodies and sinusoidal and perivenular fibrosis; sometimes periportal fibrosis can predominate (Cotran et al., 1999). The severity of

alcoholic hepatitis can differ remarkably and may lead to life-threatening liver failure (Gao & Bataller, 2011; Philips et al., 2019). Liver fibrosis is especially characterised by increased fibrogenesis, with overproduction of collagens, glycoproteins, proteoglycans and glycosaminoglycans by activated stellate cells and myofibroblasts (Stickel et al., 2017).

Liver cirrhosis is the end stage of the alcoholic liver disease or any other chronic liver disease histologically characterised by development of fibrous septa, abnormal nodular structure and changes in (micro)vascular structure of liver with formation of shunts between afferent and efferent vessels (Schuppan & Afdhal, 2008; Tsochatzis et al., 2014). Clinically decompensation of liver cirrhosis is indicated by ascites, icterus, bleeding from oesophageal and gastric varices or encephalopathy (Tsochatzis et al., 2014). Cirrhosis is frequently accompanied with infections that increase the mortality four times (Arvaniti et al., 2010). Liver dysfunction causes deficiencies of defence mechanisms, particularly due to decreased activity of endoplasmatic reticulum, reduced number and function of Kupffer cells (Thalheimer et al., 2005). The most common infections in cirrhotic patients are urinary and respiratory tract infections, spontaneous bacterial peritonitis and bacteraemia (Navasa et al., 1997). Alcoholic liver cirrhosis is a risk factor for development of hepatocellular carcinoma, co-existing smoking and obesity increasing this risk (Singal et al., 2018). Several studies and authors suggest that genetics modify the occurrence and persons susceptibility to develop alcohol use disorder as well as alcoholic liver disease (Seitz et al., 2018).

2.5.2. Pancreatic damage

Pancreatic damage caused by alcohol can cause acute and chronic alcohol-related pancreatitis. There are several co-existing pathways of alcohol induced damage to the pancreas – the direct cellular toxicity of alcohol and its metabolites, obstruction of pancreatic ducts and autodigestion (Irving et al., 2009). Oxidative metabolism in comparison with non-oxidative is low in pancreas, and nonoxidative metabolism is resulting in formation of fatty acid ethyl esters (Clemens & Mahan, 2010) responsible for activation of proinflammatory cytokines, increasing fragility of lysosomes of acinar cells and have its role in formation of fibrosis. As in liver, injury of stellate cells in pancreas is associated to tissue fibrosis.

Acute pancreatitis with its pancreatic and general complications: pancreatic or peripancreatic fat tissue necrosis and formation of pseudocysts, pleural and pericardial effusions and renal failure, has extremely high mortality (Waldthaler et al., 2010). Attacks of acute pancreatitis may precede chronic pancreatitis. The most common adverse outcomes of chronic pancreatitis include in addition to chronic abdominal pain diabetes mellitus foremost due to insulin deficiency and insulin resistance, exocrine pancreatic insufficiency and metabolic bone disease (Hart & Conwell, 2020). Histological findings typical for chronic pancreatitis are atrophy e.g. acinar tissue loss and perilobular and interlobular fibrosis, pseu-

docysts and necrosis are also more frequent in alcoholic chronic pancreatitis than in other forms of the disease (Conwell et al., 2014).

Recent systematic review and meta-analyses of published relevant literature by Singhvi and colleagues showed that approximately 40% of patients with alcoholic pancreatitis had coexisting alcoholic liver disease (Singhvi et al., 2020). A study assessing risk factors for chronic pancreatitis in North America found that smoking was implicated in less than 10% of cases while alcohol was in 53%, and two or more risk factors were present in approximately in 70% of cases (Conwell et al., 2017).

2.5.3. Heart damage

Heavy alcohol intake is related to different cardiovascular diseases including alcoholic cardiomyopathy, hypertension, coronary artery disease and arrhythmias (Klatsky, 2010).

Alcoholic cardiomyopathy is a form of acquired or secondary dilated cardiomyopathy with left or both ventricles dilatation and dysfunction, leading to heart failure. This diagnosis is established *per exclusionem* in case of chronic alcohol abuse and no other evident cause of dilative cardiomyopathy present. Different authors suggest that the alcohol consumption over 80 g daily during at least 5 years is required to diagnose alcoholic cardiomyopathy (Mirijello et al., 2017; Weintraub et al., 2017), and there is association between longer duration of alcohol abuse and symptomatic alcoholic cardiomyopathy (Piano & Phillips, 2014). The exact prevalence of alcoholic cardiomyopathy is not known, probably due to difficulties and varieties of practice in coding this disease. The number of alcoholic cardiomyopathy cases in individuals with dilative cardiomyopathy varies greatly from 3% to 47% according to different studies in America and Europe (Guzzo-Merello et al., 2014). Alcoholic cardiomyopathy is more common in men, but women may develop this condition earlier and with lower amount of alcohol used (George & Figueredo, 2011). Recent Finnish study confirmed that alcoholic cardiomyopathy remains frequently un-diagnosed *pre mortem* despite heavy drinking has been present (Hietanen et al., 2020).

Histological findings in alcoholic cardiomyopathy are nonspecific: interstitial and endocardial fibrosis of various degrees and various size of muscle cells are main changes (Cotran et al., 1999; Guzzo-Merello et al., 2014). The development of alcoholic cardiomyopathy is multifactorial and depending on individual susceptibility; cells are injured by ethanol and acetaldehyde. Pathophysiologic mechanisms contributing to development of alcoholic cardiomyopathy are oxidative stress, apoptosis, dysfunction of intracellular organelles, alterations calcium transient and myofibril calcium sensitivity, changes in myofilament structure and their function, activation of renin-angiotensin and catecholaminergic system. The genetic predisposition to development of alcoholic cardiomyopathy has also been determined (Ware et al., 2018).

Recent systematic meta-analyses of articles about chronic alcohol consumption linked to the atrial fibrillation showed that high alcohol intake increases the risk of atrial fibrillation generally and moderate intake in males (Gallagher et al., 2017), acute alcohol intake, especially binge drinking has been known as a causative factor of arrhythmias for a long time (Tonelo et al., 2013). Arrhythmias may play role in sudden unexplained death in alcohol misuse, as well. This diagnose is used in United Kingdom in case of deceased with background of chronic alcohol use, liver steatosis and no sign of cardiac abnormality or acute alcohol toxicity (Sorkin & Sheppard, 2017). The relation of excessive alcohol use and hypertension has been proved by different studies, and alcohol related hypertension is common reversible form of hypertension (Klatsky, 2010). Regular alcohol use increases blood pressure depending on dose with relative risk for hypertension 1.7 for 50 g ethanol/day and 2.5 at 100 g/day (Day & Rudd, 2019). Recent multicenter case-cohort study involving eight European countries confirmed alcohol consumption as a risk of ischemic and hemorrhagic stroke and previously known J-shaped nonlinear association between alcohol and coronary artery disease (Ricci et al., 2018).

2.5.4. Gastrointestinal tract damage

As in other organ systems, alcohol has its acute and chronic adverse effect on gastrointestinal tract. Mallory-Weiss syndrome – esophageal tears caused by vomiting occur usually after recent intake of a considerable amount of alcohol (Bujanda, 2000; Michel et al., 1980). Acute alcohol intake is also related to increase of gastroesophageal reflux caused by motility disorders and decreased lower sphincter tone of the esophagus (Franke et al., 2005). Although alcohol damages gastric mucosa, the relationship between alcohol abuse and chronic gastritis has not been clearly proven, and it has been even shown that modest alcohol consumption may have a protective effect against *Helicobacter Pylori* infection (Bujanda, 2000; Franke et al., 2005; Taylor et al., 2005). Development of acute erosive and hemorrhagic gastritis after alcohol consumption has been proven experimentally with mucosal damage being dose dependent (Knoll et al., 1998). Esophageal and gastric varices develop as complication of liver cirrhosis, as liver tissue fibrosis and increased vascular resistance lead to portal hypertension (Lesmana et al., 2020) and can result in lethal bleeding. Arakawa and colleagues according to their study of varices had come to the result that ruptured varices of fundus may cause more significant bleeding than esophageal or cardiac varices, and the exact site of rupture in esophagus is hard to determine (Arakawa et al., 2002). The effects of alcohol on acid secretion in stomach and gastric emptying are variable depending on alcohol concentration and type of alcohol ingested (Bujanda, 2000). In the intestine chronic alcohol intake causes dysbiosis and bacterial overgrowth that may contribute to increased permeability and endotoxemia with endotoxins entering liver, and in this way, alcohol caused inflammatory process of intestines contributes to liver injury (Bishehsari et al., 2017; Bujanda, 2000; Engen et al., 2015).

2.5.5. Lung damage

Chronic alcohol abuse is a risk factor for respiratory diseases including pneumonia, chronic obstructive pulmonary disease and acute respiratory distress syndrome (Kaphalia & Calhoun, 2013). A review of community acquired pneumonias found more than eight times increased risk of pneumonia in alcohol abusers with dose-response relationship (Samokhvalov et al., 2010). All types of bacterial pneumonias are more common in alcohol abusers. The pathogens, *Streptococcus pneumoniae* being the most frequent, causing pneumonia do not differ from those in the general population (Gamble et al., 2006). At the same time, it has been observed that in alcoholic patients there is increased incidence of Gram-negative pathogens, especially *Klebsiella pneumoniae*, high frequency of complications and poorer outcome (Gamble et al., 2006; Zhang et al., 2008). In the most common ways heavy alcohol consumption increases the risk of pneumonia are airways colonization with pathogens potentially associated with aspiration of gastric content, compounded by decreased mucociliary barrier of upper airways and impaired pulmonary host defenses (Kershaw & Guidot, 2008). Furthermore, infection with *Mycobacterium tuberculosis* has higher incidence in alcohol abusers, influenced by persons lower socioeconomic status and immune-altering effects of alcohol (Gamble et al., 2006). Acute respiratory distress syndrome is more likely to develop in critically ill persons with chronic alcohol use history as their lungs are more vulnerable to oxidative stress, but it is possible that other factors e.g. liver cirrhosis and aspiration are contributing in development of acute respiratory distress syndrome (Thakur et al., 2009).

2.5.6. Brain damage

Alcohol has acute and chronic adverse effects on brain causing chronic neurodegeneration and also contributing to brain injuries and stroke (de la Monte & Kril, 2014). Acute alcohol poisoning can cause fatal brain edema, also result in fatal hemorrhage in different brain regions – ventral diencephalon, mesencephalon and basal ganglia (de la Monte & Kril, 2014). Different studies have shown that in alcoholics the decrease of brain weight and volume is caused mainly by white matter loss, especially in prefrontal region, and is caused most likely by changes in myelination and microstructural integrity of axons (Harper & Matsumoto, 2005). In brain astrocytes, oligodendrocytes and synapsis are considered to be the most vulnerable to alcohol toxicity, changes in dendritic arbors have been described in autopsy material by Harper and Corbett (Harper & Corbett, 1990). Cerebellar degeneration causing ataxia in chronic alcoholics can occur with or without micronutrient deficiency (Noble & Weimer, 2014), while vermis and Purkinjé cells being most commonly affected (de la Monte & Kril, 2014). In case of severe liver damage – cirrhosis and dysfunction with failure, hepatic encephalopathy can develop. Reduced liver function causes increase of serum ammonia levels, which have neurotoxic effects (Butterworth, 2000) and neuropathological, morphological and functional changes appear in

astrocytes. Furthermore, malnutrition and alcohol's inhibitory effects of thiamine absorption, cause increased incidence of Wernicke's encephalopathy in chronic alcohol abusers (de la Monte & Kril, 2014). Brain tissue injury via inhibited metabolism occurs mostly in structures surrounding the third and fourth ventricles, aqueduct and especially in mammillary bodies (Sinha et al., 2019).

2.6. Alcohol-related biomarkers

In forensic medicine, chronic alcohol abuse is usually determined according to autopsy findings (macroscopically and on histological examination) and, if available, deceased person's medical records and preliminary data provided by the police. Acute intoxication is diagnosed by detecting ethanol in different body fluids. Besides this, there are several markers of acute as well as of chronic alcohol consumption used in clinical practice and in *post mortem* diagnostics. Determination of alcohol biomarkers is also important in other situations, e.g. verifying abstinence after confiscation of driving licence, proving alcohol abstinence objectively or excessive use in clinical aspect (Andresen-Streichert et al., 2018). Alcohol biomarkers are divided into indirect, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT) and mean corpuscular volume (MCV), and direct markers of alcohol, such as ethyl glucuronide (EtG), ethyl sulphate (EtS), phosphatidyl ethanol (PEth).

In order to verify chronic alcohol abuse, analyses of serum AST, ALT and GGT levels and mean corpuscular volume (MCV) have been widely used in clinical practice, and carbohydrate-deficient transferrin (CDT) has also been considered to be one of the conventional markers of alcohol use (Rainio et al., 2008; Andresen-Streichert et al., 2018). Above mentioned AST, ALT and GGT identify long-term (months-years) excessive alcohol use, but have low sensitivity for recent alcohol abuse (Helander, 2003). At the same time their use as diagnostic tool for alcohol consumption is problematic for their low sensitivity and specificity, except CDT (Jastrzębska et al., 2016). Increased MCV, for example, indicating folate or B12 vitamin deficiency does not differentiate the alcoholic or non-alcoholic cause of the condition (Walsham & Sherwood, 2014). Abnormalities in liver enzyme (GGT or ALT) activities may be the first signs of alcohol abuse, but at the same time they can indicate existing non-alcoholic fatty liver disease or overlapping alcoholic and non-alcoholic liver pathology (Niemelä, 2016; Tsai et al., 2012; Yki-Järvinen, 2014). The recent population binge drinking and lifestyle study showed significant increase of GGT in men and women in association with increasing frequencies of binge drinking and the same trend was observed for ALT in men; also increase of ALT and GGT was found in those with heavy drinking episodes at least once a month. In women, GGT values were significantly increased in groups with heavy drinking episodes at least once a week (Nivukoski et al., 2019). As AST is present not only in hepatocytes, but in skeletal muscle and heart tissue,

kidneys and brain, its activity elevation is often increased due to extrahepatic reasons. Interpreted together, AST/ALT ratio over two has been considered suggestive of alcoholic aetiology (Pratt & Kaplan, 2000). Elevated liver aminotransferases and AST/ALT ratios have, however, also been reported from non-alcoholic steatohepatitis patients with a high fibrosis risk as markers to monitor and evaluate changes in inflammation (Suzuki et al., 2006). *Post mortem* evaluation of AST and ALT is problematic due to usual prolonged period before autopsy – long *post mortem* interval – that causes haemolysis and leads to unreliable values of activity of mentioned liver enzymes, as it was shown in Estonian study of alcohol and premature death. GGT is less sensitive to *post mortem* interval (Fumeaux et al., 2018).

CDT is a specific, but not very sensitive biomarker. This has been the first alcohol-specific routine biomarker to indicate moderate to prolonged heavy drinking. Testing CDT reflects heavy drinking over the past week up to approximately one month with very low risk of false-positive results (Helander et al., 2016), that may occur due to rare genetic variations. It has been noted that CDT is with lower sensitivity in women (Anttila et al., 2005). CDT levels are also connected to body-mass index and smoking showing lower values among obese persons and higher in smokers compared to lean and non-smokers using comparable amount of alcohol (Nanau & Neuman, 2015). However, in *post mortem* analysis the high prevalence of haemolysis of blood samples makes CDT a less useful test. For this reason, measurement of CDT in vitreous humour is probably more valuable (Rainio et al., 2014).

EtG and EtS are the direct ethanol metabolites that are important markers of recent alcohol ingestion. EtG can be determined in different biological samples after alcohol elimination from the body and is also usable in *post mortem* diagnostics of recent alcohol use (Rainio et al., 2008). Compared to short and long-term markers it is an intermediate marker of alcohol consumption (Musshoff, 2002). In urine, EtG can be detected for up to 130 hours (3–5 days) after excessive alcohol intake, however the time is shorter with smaller amounts of alcohol (Andresen-Streichert et al., 2018; Jastrzębska et al., 2016). EtG is also detectable in hair allowing to assess alcohol intake retrospectively for a longer period than in blood and urine, and has been suggested to be used for detecting abstinence (Wurst et al., 2015). Renal dysfunction may increase EtG and EtS levels and prolong their elimination. Urine samples with bacteria present, especially *Escherichia coli*, may give falsely decreased values of EtG; EtS is considered to be less sensitive to bacterial hydrolysis (Walsham & Sherwood, 2014). Determining EtG in different *post mortem* samples has been studied by different authors (Krabseth, Mørland & Høiseth, 2014; Vezzoli, Bernini & de Ferrari, 2015), and it has proven to be quite stable to *post mortem* degradation. Studies also indicate that EtG can be used as an alcohol biomarker due to its high specificity in cases where *post mortem* ethanol formation is questioned. It has been even advised that in forensic autopsy cases with BAC below 1 g/kg, determining EtG and EtS should be recommended (Krabseth et al., 2014).

PEth is an abnormal phospholipid produced in cell membranes, erythrocytes included in the presence of alcohol. Measuring PEth levels in whole blood can be used to determine recent or current alcohol consumption and chronic alcohol abuse. According to Andresen-Streichert et al. (Andresen-Streichert et al., 2018), PEth can be determined in blood 1–2 hours after single alcohol intake for up to 12 days, and in chronic use up to 3 weeks (Wurst et al., 2015). In comparison with CDT and GGT PEth has shown higher sensitivity in determination of harmful drinking (Isaksson et al., 2011; Neumann et al., 2020). At the same time, PEth is considered to be less sensitive to small amounts of alcohol compared to EtG or EtS (Jastrzębska et al., 2016).

2.7. Coding underlying causes of death

The role of alcohol is reflected by its presence among causes of death and largely used for statistical purposes. The amount and distribution of alcohol related causes of death depends on the coding practice and availability of relevant codes of causes of death.

The validity of alcohol poisoning deaths in Estonia has been questioned (Rahu et al., 2011) and the authors concluded that there was an obvious misclassification in coding of alcohol poisoning and mental disorders due to alcohol as underlying causes of death in the Estonian Causes of Death Registry.

In a recent study from Finland it was assessed how consistently and completely the role of acute alcohol (ethanol) intake as a cause of death is reported on death certificates, how complete and specific the statistical recording of cause-of-death data on acute alcohol-induced deaths is, and how the information ultimately appears in the national mortality statistics. (Lahti et al., 2011).

Overall, a concentration-dependent association was found between forensic-toxicologically determined blood alcohol concentrations and acute alcohol-specific cause-of-death diagnoses. Based on a medicolegal re-evaluation of death certificates, acute alcohol-specific causes were found to be underreported nationally at a rate of 8%.

2.8. Deaths caused by psychotropic and other substances

Psychotropic substances

Psychotropic substances and narcotic drugs, potentially causing abuse and dependence, are divided into six schedules in Estonia and their handling is prohibited except for medical or scientific purposes.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) defines drug-related (or drug-induced) deaths as cases where people die directly due to use of illegal substances, although these often occur in combination with other substances such as alcohol or psychoactive medicines (EMCDDA, 2019). These deaths occur generally shortly after the consumption of the substance and are also known as overdoses or poisonings.

According to EMCDDA European Drug Report 2020 (EMCDDA, 2020) it is estimated that at least 8300 overdose deaths involving illicit drugs, primarily opioids, occurred in the EU in 2018 (Norway and Turkey excluded). The numbers of drug poisoning deaths had decreased in 2018 only in Sweden, Estonia and Turkey.

Drug poisoning mortality in Estonia and neighbouring countries is shown in Figure 5. In Estonia, drug poisoning mortality per 100 000 inhabitants increased from 1.6 in 1999 to 12.8 in 2012 and thereafter decreased until 3.0 in 2018. In Latvia and Lithuania, it was stable varying from 0.3 to 1.8 and from 1.0 and 3.9, respectively, over study years. In Finland, drug poisoning mortality increased slightly from 2.3 in 1999 to 4.7 in 2018. In Sweden it increased from 1.8 in 1999 to 6.9 in 2015 and thereafter decreased to 5.5 in 2018. In Norway drug poisoning mortality started to decrease from 9.0 in 2001 to 4.7 in 2017.

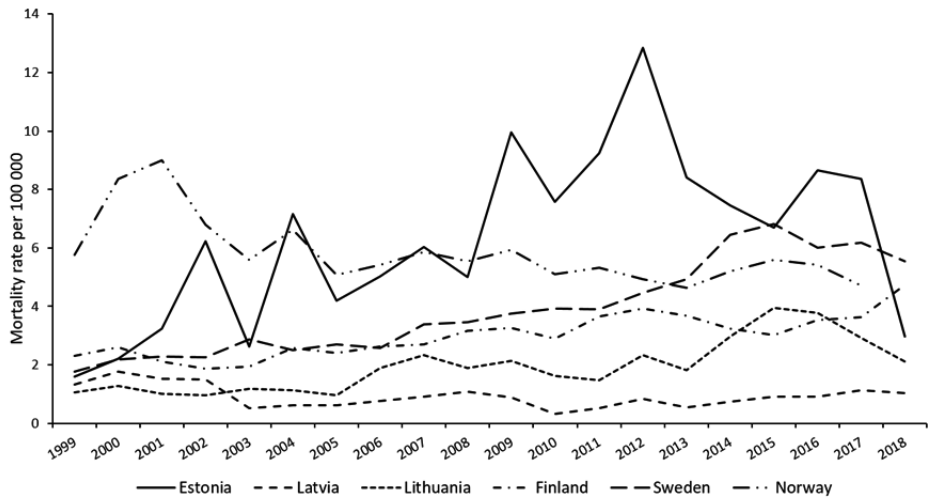


Figure 5. Drug poisoning mortality rates (crude) in Estonia and neighbouring countries, 1999–2018 (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 2019.

Morphine and its semi-synthetic and synthetic analogues, including fentanyl, are used as pain relievers and anaesthetics. In Europe, Estonia stands out as having an endemic problem with entrenched patterns of fentanyl use, including injection and associated high mortality rate (Mounteney et al., 2015). In Estonia, the number of deaths caused by fentanyl were remarkable in 2002 and 2009, an epidemic of poisonings due to other synthetic opioid 3-methylfentanyl occurred in 2005–2006 (Ojanperä et al., 2008). The use of synthetic opioids, especially fentanyl, has also been increasing in USA, where deaths caused by fentanyl doubled during 2015–2016 (Dowell et al., 2017). In most cases other drugs (often opiate, alcohol, amphetamine, cocaine or benzodiazepines) are

detected with fentanyl in poisoning cases. There is no clearly defined minimum fatal concentration of fentanyl with concentrations causing death from as low as about 0.2 ng/mL (Drummer, 2019). Autopsy findings are non-specific, and death is usually caused by the depression of central nervous system. In the review of literature (Frisoni et al., 2018) in addition to pulmonary oedema, aspiration of gastric content has been mentioned in multiple cases. According to various publications, deaths from 16 novel opioids have been reported in the last years, including acetylfentanyl, acrylfentanyl, carfentanyl etc (Drummer, 2019).

Fatalities due to misuse of fentanyl transdermal patches have been described in several countries, for example Germany, Australia, and USA, where fentanyl patches have been chewed and aspirated, as well (Carson et al., 2010). This kind of misuse has not been documented in Estonia.

Buprenorphine and methadone are used in opioid maintenance treatment and serious pain relievers. Poisonings from methadone are not frequent in Estonia comprising approximately 6.3% of all deaths from illegal drugs during 2000–2009. Methadone related deaths have been leading causes of poisoning deaths in Nordic countries. For example, in 2017 in Denmark methadone poisoning was the most frequent, although in decline, cause of death in drug addicts (Simonsen et al., 2020). From the research in Norway in 2000–2006 (Bernard et al., 2013) there were over 300 deaths caused by methadone, and most of them were combined intoxications. Finland has been unique with its high mortality from buprenorphine deaths, about half of poisoning deaths have been from buprenorphine in 2010–2014, while majority of the deceased were not receiving opioid maintenance treatment (Kriikku et al., 2018). In buprenorphine deaths benzodiazepines, usually in therapeutic concentration, and alcohol are often found in blood samples (Häkkinen et al., 2012).

According to stimulants, which are often used as recreational drugs, poisonings with amphetamine were ranking fourth in illegal-drug poisonings in Estonian study. In systematic review of persons with amphetamine abuse or misuse that included different countries of Asia-Pacific, North America and several Nordic countries showed, that compared to general population, the most highly elevated causes of death among people with regular or problematic amphetamine use were drug poisoning, homicide and suicide (Stockings et al., 2019).

Carbon monoxide

Carbon monoxide is colourless and odourless gas that is inhaled and absorbed in lungs. Common sources of carbon monoxide are cars exhaust fumes, incomplete organic materials combustion in house-fires, and gas heating appliances without proper ventilation. The diagnosis of carbon monoxide poisoning is based on elevated levels of carboxyhaemoglobin in blood samples and the symptoms of intoxication are mainly related to brain and heart (Smollin & Olson, 2010). There are specific autopsy findings in carbon monoxide poisoning (e.g. bright pink coloration of blood and viscera), and usually carboxyhemoglobin

(COHb) levels are over 50–60% in blood samples (Ruas et al., 2014). The Swedish study of fatal fire accidents in 1999–2007 (Jonsson et al., 2017) showed that there were about 99 deaths from fatal residential fires annually. This study and also Portuguese 10-year study of forensic autopsies (Ruas et al., 2014) with COHb present revealed that COHb lower levels were detected in elderly victims and in the group with thermal lesions. Especially vulnerable to carbon monoxide poisoning are considered to be persons with cardiovascular diseases and anaemia, elderly with co-morbidities, children and pregnant women (Smollin & Olson, 2010). As the manner of death, most of the carbon monoxide poisonings are accidental or suicides (Janík et al., 2017).

Medicines

In Estonia, poisoning deaths caused by medicines are quite rare and in 2000–2009 comprised 3.8% of all lethal intoxications, about one-third of them being poisonings by tricyclic antidepressants. In comparison with this, in England and Wales during 1993–2004 deaths involving antidepressants comprised about 15% of all poisoning deaths (Flanagan, 2008). The fatalities with drugs in USA in 2019 were caused, when pharmaceutical and illegal opioid preparations, miscellaneous alcohols and stimulants and street drugs excluded, in first place by acetaminophene, cardiovascular medicines (calcium antagonists and beta blockers) and various antidepressants (Gummin et al., 2020).

Tricyclic antidepressants are common in treatment of depression, and while overdosed cause most frequently hypotension, seizures and central nervous system depression up to coma, death is presumably caused by various cardiac arrhythmias, QT prolongation leading to ventricular tachycardia and cardiac arrest (Prahlow & Landrum, 2005).

The second biggest group of lethal poisonings with medicines in Estonia were intoxications with neuroleptics, or antipsychotic drugs, that are mainly used for treatment of schizophrenia but also for other psychiatric disorders and indications (e.g. sleeping disorders). Study of antipsychotic drug-related deaths in England and Wales 1993–2013 has shown that about 2.7% of the poisoning deaths of this period were related to first (mostly chlorpromazine and thioridazine) or second generation (mostly olanzapine, quetiapine and clozapine) antipsychotics (Handley et al., 2016). Overdose with antipsychotics cause different life-threatening conditions such as the depression of central nervous system, hypotension, respiratory failure and cardiac arrhythmias (Levine & Ruha, 2012).

2.9. Forensic medical system in Estonia

Estonian Forensic Science Institute (Estonian Forensic Science Institute, 2021) is a state forensic institution administered by Ministry of Justice where all forensic medical examinations, forensic autopsies included, are conducted in Estonia (Forensic Examination Act, 2021). There are 17 forensic doctors at Estonian Forensic Science Institute, some of them working part-time. Forensic

autopsies are carried out in one of four regional forensic medical departments of Estonian Forensic Science Institute (located in Tallinn, Tartu, Kohtla-Järve, Pärnu) on request of police, prosecutor or court.

According to Establishment of Cause of Death Act (Establishment of Cause of Death Act, 2021), forensic autopsy is conducted in one of following circumstances: if there is evidence of a crime; where death appears to be caused by external factors (injury, poisoning or violence) but no crime is suspected; where the state of the body makes it impossible to assign a cause of death from external inspection; or where the identity of the deceased is unknown.

In 2009–2020 there has been in total 19 528 forensic autopsies in Estonia. The number of autopsies performed in this time period fluctuated from 1429 in 2019 to 1846 in 2009 to 1429 in 2019 with the proportion of autopsied women in the range of 21–24% (Estonian Forensic Science Institute, 2021).

2.10. Brief summary

In Estonia, premature mortality, associated with excessive alcohol consumption, is high, especially in men. It is therefore important to find out the role of alcohol in causing damage to human body as well as the magnitude of it affecting population health. As has been shown in research literature, one of the ways of doing that is to carry out an autopsy-based study. Yet these are quite rare in the world as they are labour-intensive and complicated, and have not been performed in Estonia.

The present study was undertaken to fill this gap. It explores deaths caused by alcohol among working age men using an in-depth forensic autopsy analysis, explores the coding problems of underlying cause of death in presence of multiple alcohol-related pathologies and describes fatal poisonings caused by alcohol, psychotropic and other substances among general population over the last decades in Estonia.

3. AIMS OF THE STUDY

The general aim of the study was to give an evidence-based overview of deaths caused by alcohol, psychotropic and other substances, and to describe registration of alcohol-related deaths in Estonia.

The specific aims were:

1. To describe alcohol exposure, underlying causes of death and alcohol biomarkers among 25–54-year-old men in 2008–2009. (Paper I)
2. To determine the prevalence of alcohol-related organ damage and to analyse their association with alcohol biomarkers among 25–54-year-old men in 2008–2009. (Paper II)
3. To explore the coding problems of underlying cause of death in presence of multiple alcohol-related pathologies. (Paper II)
4. To analyse fatal poisonings caused by alcohol, psychotropic and other substances among general population in 2000–2009 (Paper III) and in 2010–2019.

4. MATERIAL AND METHODS

The thesis consists of two studies. Firstly, the study of premature mortality in association with alcohol exposure in working-age men deaths in Estonia subject to forensic autopsy in 2008–2009 using autopsy and proxy interview data with measured blood, urine and vitreous humour alcohol concentration, liver enzymes activities and alcohol biomarkers (Papers I, II). Secondly, the study of all poisoning deaths in Estonia from 2000–2009 using the data from autopsy reports of the Estonian Forensic Science Institute (Paper III) and updating poisoning deaths' data for 2010–2019 (data not yet published).

4.1. Study I: Premature mortality in association with alcohol exposure in working-age men (Papers I, II)

4.1.1. Study subjects

The study population consisted of all deceased males aged 25–54-years subject to forensic autopsy in whole Estonia in 2008–2009. This age band was chosen to reflect working age. Formation of the study sample is shown in Figure 6 (Figure 1, Paper I). The total number of men autopsied in this period was 2670 and 1299 of them belonged to the study age group. Forensic doctors participated in the study voluntarily. Because filling special autopsy protocol was the additional assignment only 605 autopsies of 1299 were included in the study. As 10 deaths were outside the designated age range or remained unidentified, the final number of eligible autopsies was 595 (Paper I). To investigate closer alcohol-related pathologies, attention was restricted to the 545 autopsies (Paper II) which had complete information about presence or absence of pathology in each organ system considered. In the 41 excluded cases, the status of some or all organs could not be determined due to putrefaction, major trauma or fire.

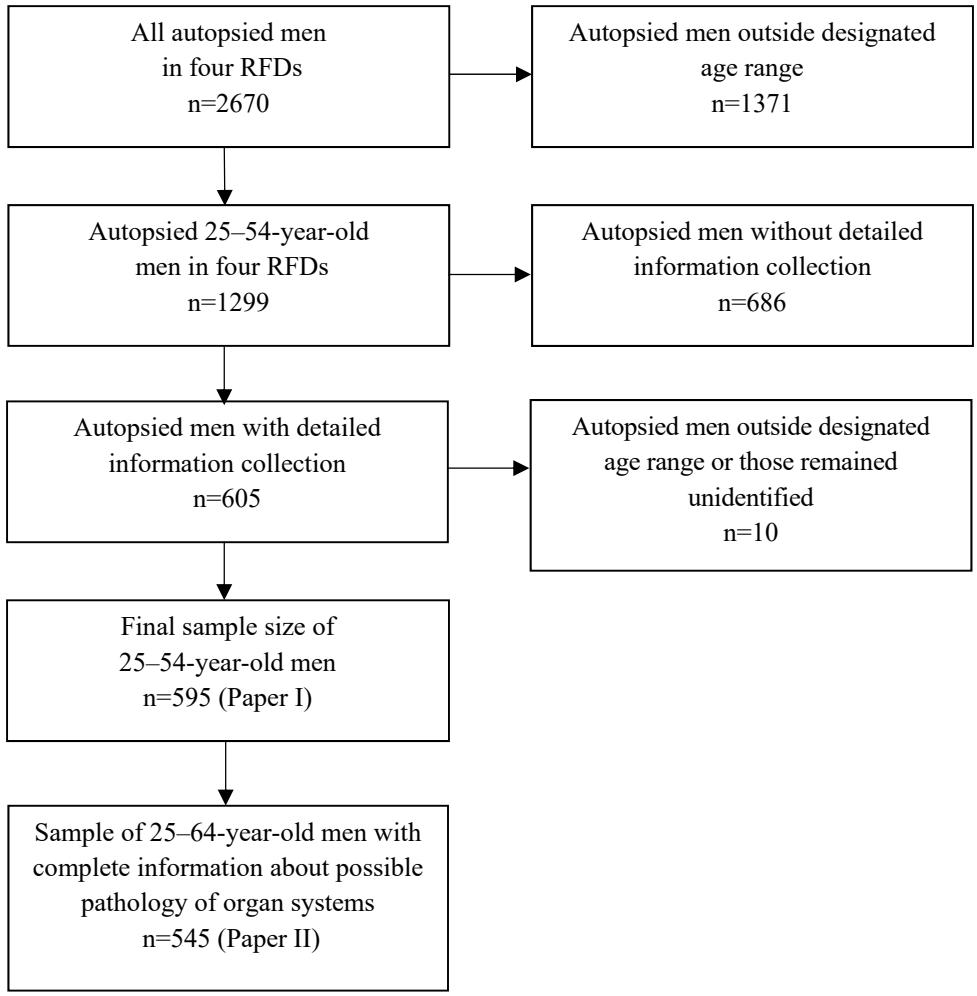


Figure 6. Flow chart of formation of study population of 25–54 men in four Regional Forensic Departments (FRDs) in 2008–2009 in Estonia.

4.1.2. Forensic autopsy related procedures

Autopsy protocol

To collect autopsy data, the enhanced autopsy protocol was implemented (Appendix 1). At autopsy, a systematic examination of the organs was undertaken, and the results were recorded by the forensic doctor using structured pro-forma. This included macroscopic evaluation of heart, kidneys, lungs, liver, pancreas, oesophagus, stomach, duodenum and brain, and histology findings of these organs.

Histological examination

Tissue sections were taken for histological examination from the parenchyma of lungs, liver, spleen, pancreas, heart, brain, stomach and both kidneys, and were fixed in buffered 10% formalin (pH 7.4) for 24 hours and embedded in paraffin wax. Histological evaluation was performed by forensic doctors and recorded in autopsy protocol.

Biological samples analysis

Ethanol concentrations in blood, urine, and vitreous humour were measured, liver enzymes – aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT) in serum were assayed, as were direct alcohol biomarkers (i.e. ethanol metabolites) phosphaditylethanole (PEth) in whole blood and ethyl glucuronide (EtG) and ethyl sulphate (EtS) in urine.

Blood sample was collected from peripheral vessels, usually from the femoral vein, using a syringe and injected into vacutainer tubes (two 10-mL EDTA and one 10-mL plain). Urine sample was taken directly from the bladder with a syringe. Vitreous humor was aspirated from both eyes using a syringe. Samples were initially refrigerated at 4^{o}C before being transferred to the forensic laboratory for processing and analysis.

One EDTA and one plain tube were centrifuged (2100 rpm) for 15 minutes at 4^{o}C and the plasma and serum collected. According to the amount of material available up to eight bar-coded cryotubes were filled for each case: two each with whole blood, plasma, serum and urine. One cryotube of each specimen was stored at -80° C.

Urine and blood alcohol concentrations (BAC) were measured by headspace gas chromatography. ALT and AST were measured using the kinetic photometric method and GGT with the kinetic colorimetric method in a Hitachi 2000. Urine and whole blood samples were sent on dry ice to the Karolinska Institutet, and several alcohol biomarkers (PEth, EtG, EtS) were measured there.

4.1.3. Autopsy-based classes of alcohol-related pathologies

As the study of premature mortality focussed on deaths related to alcohol consumption, six classes of potentially alcohol-related pathologies of the liver, pancreas, lung, stomach, oesophagus and heart were defined specially for this study. The alcohol-related pathologies were determined from a combination of gross examination of organs and histology as described in Table 1, Paper II. The presence of oesophageal varices was determined macroscopically. The presence or absence of each class of pathology was coded as a binary variable, with no intermediate gradations.

4.1.4. Interviews with proxies

For a subset of cases (n=276) who had lived in one of the five major towns of Estonia (Tallinn, Tartu, Pärnu, Kohtla-Järve, Narva) proxy interview data was collected about the drinking behaviour of the deceased.

The interview questionnaire focussed on collecting information on the socio-demographic characteristics and health behaviours of the deceased (Table 7, Paper I) (Appendix 2). Particularly detailed information was collected about alcohol consumption, with a reference period of the year preceding the man's death. Within two weeks of the forensic autopsy protocols and samples arriving in Tartu, details of next-of-kin were sought from the forensic departments. After a minimum of two months following the death, the deceased's next-of-kin were contacted. Following a brief explanation about the study, they were asked if they, or someone else would be willing to be interviewed about the deceased's life. Respondents signed informed consent. In order to maximise the validity of the information provided, pre-defined criteria were used to select the best informant – family member who had had daily contact with the deceased during the last year before his death – when several were available. If the next-of-kin was not the best proxy, or refused to be interviewed, contact was then made with another proxy. The process of selection of subjects for proxy informant interview is shown in Figure 2, Paper I. Of 276 deceased men eligible, for 7% it was not possible to find out whether they had had a family, and of those for whom it was possible to determine whether they had a family, 7% had lived alone. Of the remaining 239 deceased men, 226 had families eligible for interview. Of these, a proxy interview was conducted for 169 (75%), the remainder either refusing (20%) or did not respond (5%), resulting in a response rate of 61%. The distribution of the relationship of the proxy to the subject was wife/girlfriend/partner (n=47), parent (n=46), mother (n=35), father (n=11), sister (n=16), other close relative (n=12), other companion (n=12), brother (n=11), son (n=11), daughter (n=7), friend (n=7).

4.1.5. Coding underlying cause of death

The underlying cause of death, and up to three associated causes of death as determined by the forensic pathologist was recorded and coded according to the

10th revision of the International Classification of Diseases (ICD-10). To compare underlying causes of death certified by forensic doctors and by the specialists of the Estonian Causes of Death Registry, and to find out problems in coding, the underlying cause of death for each subject was obtained, as assigned and coded by the Estonian Causes of Death Registry (Paper II). In the analysis of these data, focus was on the following underlying causes: alcohol dependence syndrome (ICD-10 F10.2), alcoholic cardiomyopathy (I42.6), alcoholic liver disease (K70) and acute alcohol poisoning (X45), cardiovascular diseases (I00–I99, except I42), other cardiomyopathies (I42, except I42.6), digestive diseases (K00–K93, except K70), respiratory diseases (J00–J99), and external causes (V01–Y98, except X45).

4.1.6. Statistical analysis

Paper I. Descriptive statistics were mainly presented as in frequency tables. Medians with interquartile ranges (IQR) or ranges were used for presenting the results of biomarkers. Fisher or chi-squared test was used for assessing the association between different characteristics (age, cause of death, BAC) and inclusion/exclusion to the study or whether a proxy interview was obtained. Comparing biomarker levels between different proxy-reported alcohol drinking frequencies, t-test or linear regression was used with log transformed biomarker values. The data were analysed using the statistical package Stata 10.

Paper II. Frequency tables and cross-tabulations were examined, with means, standard deviation and medians of biomarkers as appropriate. The association between biomarker levels and each class of pathology was assessed using linear regression with log transformed biomarker values. The non-parametric Cuzick test for trend across ordered groups was used for identifying the relationship between frequency of drinking and pathology classes among drinkers. Logistic regression was used to estimate the strength of association between cause of death and the number of positive pathology classes adjusted for age. For this analysis, cause of death was dichotomised into alcohol-related (I42.6, K70, F10.2, X45) and other (all other) causes. Data were analysed using Stata 11.

4.2. Study II: Poisoning deaths in Estonia (Paper III)

4.2.1. Data collection

The study of fatal poisonings was based on data (age, sex, cause of death, toxicology results) collected from all the autopsy reports of the Estonian Forensic Science Institute and from the Department of Forensic Chemistry during the period 2000–2009 (Paper III) and 2010–2019 (data for the latter period not published). All cases where the underlying cause of death was poisoning (n=4132 and n=2822 respectively) were included and ICD codes T according to

substance (ethanol, illegal drugs, medicines, other alcohols, carbon monoxide, other and unknown) were used.

4.2.2. Determination of alcohol, psychotropic and other substances in biological samples

During the autopsy, samples of blood, urine and humour vitreous were routinely collected. Blood alcohol concentration was measured in all cases; bodies in a state of decomposition and skeletisation and patients who had been hospitalised for a long period were excluded. Psychotropic and other substances were analysed in all traffic accidents, when a special request was made by the police or in cases where the forensic doctors suspected drug poisoning. Alcohol was determined using headspace gas chromatography. The presence of legal and illegal drugs in the body was identified in a variety of laboratory procedures: drug screening by immunoassay; urine screening tests were performed for amphetamine, metamphetamine, opioids (heroin/morphine), methadone, cocaine, marijuana, tricyclic antidepressants, benzodiasepines, barbiturates and fencyclidine; then all positive tests were confirmed as well as for detecting other synthetic drugs (not detectable with screening tests) using gas chromatography and mass spectrometry. Of all 4132 poisoning cases, blood and urine were analysed at the Department of Forensic Chemistry in 4042 cases; in the other 90 cases, poisoning was diagnosed at the hospital.

4.2.3. Statistical analysis

Paper III. Results were presented in frequency tables. Chi-square test was used to assess differences between dichotomous variables. Mann-Kendall trend test was used to analyse changes in numbers of poisonings over the years. A p-value of <0.05 was considered statistically significant. Data were analysed using Stata 9.

4.3. Ethical approval

The Estonian Forensic Study of Alcohol and Premature Death was approved by the Ethics Review Committee on Human Research at the University of Tartu, Estonia (No. 154/22, 20.11.2006) and the processes for handling personal data were registered with the Estonian Data Protection Inspection. The study was undertaken with the full support of the Estonian Forensic Science Institute that has sole statutory responsibility for forensic autopsies in Estonia.

5. RESULTS

5.1. Alcohol exposure, alcohol biomarkers and underlying causes of death (Paper I)

Alcohol exposure

The proxy reported prevalence of drinking of deceased men at least several times in the past year was 84.0% and in the past month 53.8% (Table 2; Table 7, Paper I). Over half (56.2%) of men had been drinking almost every day or 1–4 times per week in the year before death. Prevalence of drinking surrogates at least several times in the past year was 10.7%.

Table 2. Consumption of any type of alcohol (including surrogates) of the deceased men based on information provided by a proxy informant

Characteristics	Number	%
Drank alcohol at least several times in the year before death*		
Yes	142	84.0
No	18	10.7
Do not know	9	5.3
Drank alcohol at least several times in the month before death *		
Yes	84	53.8
No	45	28.8
Do not know	22	14.1
Missing answer	5	3.2
Frequency of drinking any alcohol during last year		
Every day/almost every day	48	28.4
1–4 times per week	47	27.8
3 times per month or less frequently	47	27.8
Never/almost never	18	10.7
Do not know	9	5.3
Drank surrogates at least several times in the year before death		
Yes	18	10.7
No	127	75.1
Do not know	21	12.4
Missing answer	3	1.8
Total	**169**	**100**

*This question was asked when deceased man ever drunk alcohol in his life at least a few occasions (n=156).

Underlying causes of death

Of the 595 deaths, the largest group of deaths were those attributed to external causes (66.4%), followed by deaths from diseases (30.9%) (Table 3; Table 4, Paper I)). The most frequent diagnoses in the group of deaths from external causes were intentional self-harm (25.6%), accidental poisoning by and

exposure to noxious substances (alcohol poisoning excluded) 13.4% and alcohol poisoning 11.4%. Deaths due to diseases of the circulatory system comprised nearly one in five of all deaths and over a half of deaths from diseases (54.9%), of which ischaemic heart disease was the major single component. Alcoholic liver disease accounted for most of the deaths (81.5%) from diseases of the digestive system. The causes of death directly related to alcohol (alcohol poisoning, alcoholic liver disease, alcoholic cardiomyopathy) comprised 15% of all deaths.

Table 3. Distribution of deaths included in the study by underlying cause of death

Cause of death by major groups (ICD–10 code)	**Number**	**%**
Diseases	**184**	**30.9**
Circulatory system (I00-I99)	*101*	*54.9*
Ischaemic heart diseases (I20-I25)	42	41.6
Alcoholic cardiomyopathy (I42.6)	3	3.0
Cardiomyopathy (I42, excl I42.6)	6	5.9
Cerebrovascular diseases (I60-I69)	14	13.9
Other diseases of the circulatory system	36	35.6
Digestive system (K00-K99)	*54*	*29.3*
Alcoholic liver disease (K70)	44	81.5
Cirrhosis and fibrosis (K74)	1	1.9
Other diseases of the digestive system	9	16.7
Other diseases	*29*	*15.8*
Unknown causes (R95-R99)	**16**	**2.7**
External causes (V01-Y98)	**395**	**66.4**
Transport Accidents (V01-V99)	38	9.6
Falls (W00-W19)	23	5.8
Accidental drowning and submersion (W65-W74)	28	7.1
Inhalation of gastric contents or food causing obstruction of respiratory tract (W78-W79)	34	8.6
Alcohol poisoning (X45)	45	11.4
Accidental poisoning by and exposure to noxious substances (X40-X49, excl X45)	53	13.4
Intentional self-harm (X60-X84)	101	25.6
Assault (X85-Y09)	30	7.6
Exposure to excessive natural cold (X31)	10	2.5
Event of undetermined intent (Y10-Y34)	20	5.1
Other external causes	13	3.3
Total	**595**	**100**

The distribution of deaths by underlying cause of death is shown in Table 4, Paper I. BAC levels of ≥ 0.2 mg/g were detected in 34% of circulatory diseases, 33% digestive diseases and 67% external causes.

Alcohol biomarkers

Alcohol concentration and its biomarker levels are shown in Table 4. All tests gave results with very wide ranges, but the medians were considerably higher than the corresponding reference intervals and a large proportion tested positive for recent drinking. About 80% of the whole blood samples and urines examined tested positive for total PEth (>0.7 µmol/L) and EtG/EtS, respectively. The values of AST and ALT had extremely wide ranges, consistent with a variable degree of *post mortem* haemolysis and autolysis of hepatocytes and, for AST, myocytes.

Table 4. Alcohol concentration and its biomarker levels

Biomarker, units	Autopsies (n= 595)			
	Number	%	Median	Range
Ethanol (blood), mg/g	564	94.8	0.78	0–6.59
Ethanol (urine), mg/g	437	73.4	1.51	0–6.14
AST (serum), U/L	364	61.2	1014	59–28790
ALT (serum), U/L	364	61.2	792	8–42580
GGT (serum), U/L	365	61.3	92	15–1884
PEth (blood), µmol/L	245	42.2	9.8	0–121.5
EtG (urine), mg/L	157	26.4	79	0–2699
EtS (urine), mg/L	157	26.4	22	0–480

Median values of the various alcohol biomarkers by proxy reported drinking behaviour are shown in Table 8, Paper I. Median blood alcohol concentration was only raised substantially among daily drinkers. A more graded association was apparent for PEth in whole blood and EtS and EtG in urine, with negative or very low levels in reported abstainers. However, for a proportion of subjects where the proxy reported that the man had not drunk in the last month the biomarkers were clearly positive, suggesting proxy underreporting. The liver enzymes AST and ALT display a very wide range, there was a significant association between frequency of drinking alcohol and GGT, although this was mainly due to elevated levels among daily drinkers.

5.2. Prevalence of alcohol-related organ damage and its association with alcohol biomarkers (Paper II)

Alcohol-related organ damage

Alcohol-related pathologies were determined from a combination of gross examination of organs and histology. The most common pathology was that affecting the liver, with 60.5% of deaths showing evidence of steatosis, fibrosis or cirrhosis (Table 5; Table 1, Paper II). Pancreatic, lung or gastric pathology

was detected in 14.1%, 18.6% and 17.5% of all cases, respectively. Evidence of damage to the heart, in the form of cardiomyopathy was much less frequent (4.9%), and oesophageal varices (1.4%) were only rarely found.

Table 5. Autopsies (n, %) with each form of potentially alcohol-related organ damage

Organ	Type of potentially alcohol-related pathology	Positive finding	
		N	% (95% CI)
Liver	Focal/diffuse steatosis, complete/incomplete fibrosis, complete/incomplete cirrhosis	335	60.5 (56.3–64.6)
Pancreas	Acute and chronic pancreatitis	78	14.1 (11.3–17.3)
Lung	Pneumonia and/or aspiration of gastric content	103	18.6 (15.4–22.1)
Stomach	Gastritis	97	17.5 (14.4–20.9)
Oesophagus	Varices (determined macroscopically)	8	1.4 (0.6–2.8)
Heart	Dilative (and alcoholic) cardiomyopathy	27	4.9 (3.2–7.0)

Overall, 75% of the subjects showed evidence of one or more classes of pathology, and 32% had evidence of two or more classes. Dividing all study cases into two age groups (25–44, 45–54) approximately 65 % of men were in older age group and the presence of higher number of pathology classes increased with age (Table 2, Paper II). The prevalence of two or more pathologies was significantly higher among older (45–54 years) men. Table 5, Paper II shows the frequencies of all pairwise combinations of potentially alcohol-related pathologies. Within each class of non-liver pathology, there was a high frequency of liver pathology, while only minority of those with liver pathology showed a second type of pathology.

Association of alcohol-related organ damage and alcohol biomarkers

Table 6 (Table 3, Paper II) shows the relationship between levels of the various biomarkers of recent (ethanol, EtG, EtS) or heavy (PEth, GGT) drinking and the number of positive pathology classes.

Table 6. Mean and median levels of alcohol and its biomarkers or alcohol-induced damage by number of potentially alcohol-related classes of pathology

Biomarker	Statistics	Number of positive classes of pathology					Total
		0	1	2	3+	P-value*	
Ethanol in blood, mg/g	Mean	1.19	1.55	1.35	1.07	0.376	1.37
	SD	1.29	1.61	1.69	1.44		1.55
	Median	0.73	1.36	0.41	0.00		0.74
	N	138	226	127	43		534
Ethanol in urine, mg/g	Mean	1.57	2.11	1.84	1.54	0.860	1.86
	SD	1.61	1.91	1.99	1.84		1.86
	Median	1.15	2.47	0.98	0.00		1.63
	N	114	171	102	25		412
Ethanol in vitreous humour, mg/g	Mean	1.02	1.83	1.81	1.54	0.332	1.60
	SD	1.45	1.87	1.95	1.92		1.81
	Median	0.00	2.05	1.49	0.00		0.61
	N	37	67	31	17		152
GGT in serum, U/L	Mean	90.60	180.90	236.23	283.72	0.001	182.02
	SD	84.02	259.44	302.79	250.83		248.01
	Median	55.00	89.00	128.50	197		92.00
	N	86	145	88	32		351
PEth in blood, µmol/L	Mean	7.23	17.03	16.14	25.10	0.001	15.23
	SD	9.68	20.75	15.41	23.21		18.30
	Median	3.06	9.72	12.14	17.95		9.75
	N	55	104	58	20		237
EtG in urine, mg/L	Mean	105.00	377.52	331.61	349.79	0.033	288.13
	SD	225.82	559.16	593.89	398.19		500.71
	Median	12.70	130.50	82.75	216.30		70.50
	N	41	60	38	8		147
EtS in urine, mg/L	Mean	24.01	75.72	62.88	56.99	0.043	56.96
	SD	52.18	102.71	103.73	62.67		91.56
	Median	3.70	35.35	23.10	40.40		20.00
	N	41	60	38	8		147

*P-value for trend in means of log biomarker values. Numbers of autopsies with biomarker concentrations varied by biomarker type as biomarkers could not be measured in all cases (material was hemolysed or putrefied).

Table 7. Frequencies (%[a]) of different potentially alcohol-related classes of pathology found at autopsy by underlying cause of death assigned in the Estonian Causes of Death Registry

Cause of death (ICD-10 code)	Class of pathology						Deaths[b]
	Liver n, %, 95% CI	Pancreas n, %, 95% CI	Lung n, %, 95% CI	Stomach n, %, 95% CI	Oesophagus n, %, 95% CI	Heart n, %, 95% CI	
Explicitly alcohol related end-organ damage (I42.6, K70)	7 100.0 59.0–100.0[c]	5 71.4 29.0–96.3	2 28.6 3.7–71.0	1 14.3 0.4–57.9	2 28.6 3.7–71.0	2 28.6 3.7–70.9	7
Alcohol dependence syndrome (F10.2)	34 100.0 89.7–100.0[c]	12 35.3 19.7–53.5	4 11.8 3.3–27.5	7 20.6 8.7–37.9	0 0.0 0.0–10.3[c]	4 11.8 3.3–27.5	34
Acute alcohol poisoning (X45)	35 79.5 64.7–90.2	3 6.8 1.4–18.7	8 18.2 8.2–32.7	11 25.0 13.2–40.3	1 2.3 0.1–12.0	3 6.8 1.4–18.7	44
External causes (V01–Y98, except X45)	160 48.5 43.0–54.0	33 10.0 7.0–13.8	65 19.7 15.5–24.4	59 17.9 14.0–22.4	1 0.3 0.0–1.7	10 3.0 1.5–5.5	330
Diseases of the circulatory system (I00–I99, excluding I42)	61 69.3 58.6–78.7	12 13.6 7.2–22.6	6 6.8 2.5–14.3	7 8.0 3.3–15.7	1 1.1 0.0–6.2	1 1.1 0.0–6.2	88
Other cardiomyopathies (I42 excluding I42.6)	3 75.0 19.4–99.4	1 25.0 0.6–80.6	1 25.0 0.6–80.6	2 50.0 0.6–80.6	0 0.0 6.8–93.2	3 75.0 0.0–60.2[c]	4
Diseases of the digestive system (K00–K93, excluding I42.6)	11 25.0 13.2–0.3	7 15.9 6.6–30.1	2 4.5 0.6–15.5	3 6.8 1.4–18.7	2 4.5 0.6–15.5	2 4.5 0.6–15.5	44
Diseases of the respiratory system (J00–J99)	10 76.9 46.2–95.0	1 7.7 0.2–36.0	13 100.0 75.3–1000[c]	2 15.4 1.9–45.4	1 7.7 0.2–36.0	1 7.7 0.2–36.0	13
All other causes	9 75.0 42.8–94.5	3 25.0 5.5–57.2	2 16.7 2.1–48.4	4 33.3 9.9–65.1	0 0.0 0.0–26.5[c]	1 8.3 0.2–38.5	12

[a] % of deaths in each underlying cause group with specified pathology; [b]Number of deaths in each underlying cause group. [c]One-sided, 97.5% CI.

The analysis of relationship between levels of various biomarkers of recent (ethanol, EtG, EtS) or heavy (Peth, GGT) drinking and the number of positive pathology classes did not show associations with the mean ethanol concentration in blood, urine, or vitreous humour. For PEth and GGT in blood there were significant trends between the biomarker levels and number of pathology classes (Table 6). Although there were significant trends for EtG and EtS in urine, the main difference was between those without versus with any evidence of alcohol-related pathology.

Table 7 (Table 6, Paper II) shows the frequency of each pathology class according to the single underlying cause of death, as assigned in Estonian Causes of Death Registry. As expected, liver pathology was found in all diagnoses of explicitly alcohol related end-organ damage and of alcohol dependence, and in the vast majority of diagnoses of acute alcohol poisoning and diseases of the digestive system (Table 7). For the causes of alcohol related end-organ damage and of alcohol dependence, the frequency of pancreas pathology was also high (71% and 35%, respectively). There was a relatively high percentage of lung pathology (18%) in deaths from acute alcohol poisoning. As for deaths from diseases of the circulatory system, an intriguing finding was that more than two thirds of cases had evidence of potentially alcohol-related liver pathology. In deaths with an underlying cause of diseases of the digestive system, 92% had liver pathology and 58% pancreas pathology. As for deaths from respiratory causes, 77% had liver pathology. In external causes of death, liver and lung pathology were the most frequent, identified in ~50% and ~20% of cases.

The age-adjusted odds ratio of dying from an alcohol-related cause versus from other causes was 4.8 (95% CI 1.8–12.5) for those with one positive pathology class, and 6.7 (95% CI 2.6–17.7) for those with two or more classes, relative to those without positive classes of pathology.

5.3. Coding the underlying cause of death in presence of multiple alcohol-related organ damage (Paper II)

In Estonia, forensic doctors usually do not code the underlying cause of death on the death certificate, the causes are coded at the Estonian Causes of Death Registry. In the presence of multiple alcohol-related pathologies at autopsy, it is often a challenge to choose one as the underlying cause of death, so forensic doctors prefer to use broad diagnosis ‘chronic end-organ damage by alcohol’. These deaths (n=34, Table 7) have been assigned by the Statistical Office as having the underlying cause of F10.2 (Mental and behavioural disorders; alcohol dependence), that is a diagnose determined clinically and not suitable for *post mortem* cases. As liver is the most frequently damaged organ in multi-organ alcohol damage death cases, code K70 (Alcoholic liver disease), although always not accurate, is preferred by forensic doctors.

5.4. Fatal poisonings caused by alcohol, psychotropic and other substances (Paper III)

In 2000–2009, the number of all autopsies performed at the Estonian Forensic Science Institute (EFSI) was 28 970, 14.3% (n=4132) of them were poisonings. Distribution of poisoning deaths in 2000–2009 is shown in Table 8 (for detailed distribution of poisonings see Table 1, Paper III). In this period, ethanol was the most frequent cause of death (35.1%, n=1449), followed by carbon monoxide (27.9%, n=1151) and poisonings with illegal drugs (21.5%, n=888). In total, 284 poisonings (6.9%) from other alcohols were recorded. Over the study period, the number of alcohol poisonings decreased significantly ($p<0.05$). In total 54.4% of cases were mono-drug poisonings, mostly caused by ethanol. Poly-drug poisonings were frequent in carbon monoxide and illegal drug-related poisonings. Combined poisoning was diagnosed rarely (3.1% of all cases) in cases of poisoning with substances of a similar toxicity, as well as in cases where two or more substances were determined to be at potentially lethal concentrations or where none of the drugs was at a lethal concentration but their coalitive effect was toxic.

Table 8. Number of poisoning deaths caused by alcohol, psychotropic and illegal drugs in Estonia, 2000–2009

Substance	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	Total
Ethanol	200	221	140	173	176	120	108	138	97	76	1449
Illegal drugs	32	43	100	49	121	70	89	119	108	157	888
Medicines	7	15	21	12	18	19	16	15	16	16	155
Other alcohols	25	78	25	16	15	31	46	24	8	16	284
CO	106	132	109	150	122	135	141	115	84	57	1151
Combined	22	24	21	4	0	7	16	14	10	12	130
Other/unknown	4	7	16	5	7	7	5	9	6	9	75
Total	396	520	432	409	459	389	421	434	329	343	4132

In 2010–2019, the number of all autopsies performed at the EFSI was 16,078, 17.6% (n=2822) of them were poisonings. In these years 2201 (78%) male and 621 (22%) female fatalities were recorded. The distribution of poisoning deaths in 2010–2019 is shown in Table 9. In this period, illegal drugs were the most frequent cause of death (37.2%, n=1050), followed by ethanol (29.5%, n=832) and carbon monoxide (16.3%, n=459). In total, 178 poisonings (6.3%) from other alcohols were recorded. Over this period, number of poisonings with illegal drugs decreased significantly ($p<0.05$), but the number of ethanol poisonings remained quite stable being the highest in 2012 (n=105) and the lowest in 2016 (n=61).

Table 9. Number of poisoning deaths caused by alcohol, psychotropic and illegal drugs in Estonia, 2010–2019

Substance	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Total
Ethanol	93	81	105	77	85	92	61	69	83	86	832
Illegal drugs	120	128	183	121	106	87	113	118	45	29	1050
Medicines	18	9	17	13	15	14	17	23	20	16	162
Other alcohols	13	34	28	23	14	12	19	15	11	9	178
CO	67	70	44	37	40	51	42	43	38	27	459
Combined	5	11	6	11	14	14	11	2	8	0	82
Other/unknown	8	5	10	4	6	7	5	3	3	8	59
Total	324	338	393	286	280	277	268	273	208	175	2822

Illegal-drug poisonings

Illegal drug-related deaths comprised 21.5% of all poisoning deaths in 2000–2009, poisonings with 3-methylfentanyl (45.7%) and fentanyl (19.6%) ranking highest (total number 580), followed by morphine/heroin (10.8%), amphetamine (7.2%) and methadone (6.3%). The number of deaths caused by fentanyl was remarkable in 2002 and 2009. An epidemic of poisonings due to 3-methylfentanyl was seen in Estonia between 2004 and 2008 (Figure 2, Paper III).

Of all illegal-drug poisonings, 789 involved male fatalities and 99 involved female fatalities; the majority of the deceased (n=764) were in the 16–34 age group. The average age of those who died due to illegal drug poisoning was 28.1 for men and 30.3 for women. Gender and age distribution were similar for all years.

Fatal illegal-drug poisonings tended to be poly-drug poisonings i.e. poisonings due to one drug and alcohol or to two or more drugs with or without alcohol (73%). The other substance in most cases was ethanol. Table 2, Paper III shows the distribution of illegal drug poisonings based on number of drugs and other substances (ethanol and other alcohols) detected.

Blood ethanol concentration was below 0.2 mg/g in 61.0% cases, which shows that the victims did not consume alcohol prior to death. The biggest group of drug abusers had a BAC of 0.50–1.50 mg/g. 3-methylfentanyle and fentanyl were often combined, possibly from production of 3-methylfentanyle from fentanyl in illegal laboratories. Most commonly, illegal drugs were combined with benzodiazepines and amphetamine, followed by methadone and opiate alkaloids (Table 10; Table 4, Paper III). In poisonings with illegal drugs, medicines most frequently found in the blood were non-steroid inflammatory drugs, narcotic analgesics and tricyclic antidepressants.

Table 10. Combinations of the main lethal illegal drug with other illegal drugs detected

Main drug	Co-found drugs									
	Fentanyls	**Opiate alkaloids**	**Methadone**	**Barbiturates**	**Benzo-diasepines**	**MDMA**	**Amphetamines**	**Cocaine**	**Cannabis metabolites**	**GHB**
3-methyl-fentanyls	10	16	21	10	63	13	65	3	15	0
Fentanyls	0	10	9	2	34	2	6	0	5	0
Methadone	0	6	0	5	20	0	1	0	1	0
Opiate alkaloids	1	0	3	3	20	1	4	0	1	0
Barbiturates	0	1	0	0	10	0	0	0	1	0
Benzodiasepines	0	0	0	0	0	0	0	0	0	0
Amphetamines	3	12	7	5	12	4	2	0	2	0
MDMA	0	0	0	0	0	0	0	0	1	0
Cocaine	1	1	0	0	2	0	0	0	0	1
GHB	0	0	0	0	0	1	0	0	0	0

6. DISCUSSION

This research provided a unique overview of the alcohol exposure, underlying causes of death, existence and the extent of pathologies and alcohol biomarkers detected at forensic autopsy among working-age men. To complement, it also described fatal poisonings caused by alcohol, psychotropic and other substances among general population over two decades in Estonia.

6.1. Alcohol exposure, alcohol biomarkers and underlying causes of death

Alcohol exposure

The hazardous way of drinking of Estonian working aged men in the sample was revealed by proxy reported prevalence of drinking of the deceased. As was expected, due to the nature of the study sample which was based on forensic autopsies, over half of the men had been drinking almost every day or several times per week. Excessive exposure to alcohol was also confirmed by the fact that in 55% of the deceased had BAC 0.2 mg/g or over, which is the legal limit for driving a vehicle in Estonia. For example, in a series of routine autopsies in Norway in 1973–1992, 48% of autopsies had a BAC 0.5 mg/g or over (Nordrum et al., 2000). In Sweden, 39% of the blood-tested cases were positive for alcohol in 1992–1996 (Sjögren et al., 2000) and in Finland, there were about 38% of alcohol positive deaths (BAC 0.5 mg/g or more) based on the study of forensic autopsies in 2005 (Lahti et al., 2011). Similarly, results of *post mortem* toxicology gathered for six years in Western Switzerland revealed, that for 25% of cases BAC exceeded 0.10 g/kg (Lefrancois et al., 2021).

Furthermore, approximately 10% of working age men in study population had drank surrogates for several times in the year before death. This is supported by findings from the Estonian survey from 2006 where the prevalence of reported surrogate consumption was 3.5% in men aged from 35 to 54 in a population-based sample (Pärna & Leon, 2011).

Having detected high alcohol exposure among our study sample, we were able to proceed and examine very important associations with novel biomarkers, organ pathology, and causes of death. The results of these investigations are discussed below.

Alcohol biomarkers

The results of this study have shown that several of the novel alcohol biomarkers appeared to perform well in *post mortem* samples. PEth in blood, EtG and EtS in urine showed high specificity *post mortem* having zero or low levels in never and almost never drinkers. EtG and EtS as markers of recent alcohol intake could be used more widely in specific cases where recent *pre mortem* alcohol intake is probably accounted to death e.g. sudden cardiac death, cardiac

arrhythmias, and accidents. As EtS is less susceptible to *post mortem* degradation and synthesis than EtG (Helander et al., 2007; Helander & Dahl, 2005; Walsham & Sherwood, 2014), it may be the preferable biomarker in *post mortem* toxicology. However, the results of biomarkers should still be interpreted carefully, as the risk of *post mortem* elevation remains. EtG, that has been proven to be quite stable to *post mortem* changes (Krabseth et al., 2014; Vezzoli et al., 2015), showed some extremely high values in our study. Liver enzymes ALT and AST showed a wide range of values and proved to be of no value in *post mortem* diagnostics, whereas GGT showing chronic alcohol consumption causing liver cirrhosis and fibrosis was less affected by *post mortem* interval, haemolysis and hypoxia. Determining GGT at autopsy is still of no particular use, as the elevation is not specific to alcohol induced liver pathology.

Underlying causes of death

The distribution of underlying causes of death among working aged men in Estonia was approximately two-thirds due to external causes and one-third caused by diseases. Of external causes, suicides (25.6%) and poisonings (24.8%) were prevalent. Clear majority of deaths from external causes is specific to the sample from forensic medicine practice as all above-mentioned deaths belong to forensic autopsy according to Estonian laws (Establishment of Cause of Death Act, 2021). Yet the choice of forensic autopsies for the study is not a limitation as they provide a sample that presents high numbers of alcohol related deaths.

Half of the deaths from diseases were caused by circulatory diseases, foremost by ischaemic heart disease, followed by diseases of digestive system, mainly alcoholic liver disease (81.5%). This confirms high mortality from cardiovascular diseases in our study group, but also raises the question whether alcoholic cardiomyopathy, being foremost a clinical diagnose, can be underdiagnosed and ischaemic heart disease overdiagnosed especially regarding this age group (young and middle-aged). There are also cases where serious cardiovascular pathology was found at autopsy with co-existing high BAC level (over 3.0 mg/g), that leaves it to forensic doctor to decide if the underlying cause of death is a disease or alcohol poisoning.

6.2. Prevalence of alcohol-related organ damage and the association with alcohol biomarkers

Alcohol-related organ damage

In this study, alcohol-related pathologies were very common, that indicates the harmful alcohol consumption of 25–54-year-old men subject to forensic autopsy. Also, this study confirmed that the risk of developing multi-organ damage was related to frequent alcohol drinking. It was most concerning that only quarter of these deaths had no alcohol-related pathology of internal organs detected. The prevalence of two or more alcohol-related pathologies was found

in 32% of deceased, and it increased with age, being significantly higher among the older men group (45–54 years). Of different organs, liver was most often (in about 60% of cases) damaged, and only minority of those with liver damage had evidence of alcohol injury to other organs. At the same time the majority of cases with potentially alcohol induced pathology of other organs studied (lung, stomach, pancreas, oesophagus and heart) had coexisting liver damage. This suggests that liver is the main organ affected by alcohol as has been shown in previous research (Cederbaum, 2012; Rocco et al., 2014), also injured by bioactive products of ethanol.

Acute or chronic pancreatitis was present in approximately 14% of cases. Fast process of *post mortem* autolysis of this organ makes the evaluation of pathological changes more difficult. Possible alcohol-related changes in lungs, as pneumonia or aspiration of gastric content were detected in 18.6% and changes in stomach and oesophagus in 18.9% of cases.

In the current study, the presence of heart damage indicating alcohol injury in form of dilative cardiomyopathy was rare, only in 5% of cases. This suggests that *post mortem* diagnosis of alcoholic cardiomyopathy can be challenging due to nonspecific histological finding and lacking of previous medical documentation or clinical diagnosis. Furthermore, over two-thirds of deaths from diseases of cardiovascular system (alcoholic cardiomyopathy excluded) had coexisting potentially alcohol caused liver pathology that raises the question of the possible heart damage foremost by alcohol.

The reason why an individual heavy drinker develops alcohol damage of particular organ or organs is not fully understood, but studies have already suggested that genetics modify persons susceptibility to develop alcohol use disorder and alcoholic organ damage (Seitz et al., 2018; Ware et al., 2018).

In the group of deaths from external causes, the prevalence of alcohol caused organ-pathologies was moderately high. This suggests that the pattern of alcohol consumption that caused the damage of internal organs may also have increased risk of deaths from external causes. Moreover, high proportion of deaths from acute alcohol poisoning had also accompanying organ-pathologies, revealing that poisoning deaths mostly occurred in regular alcohol users.

In this study the presence of alcohol induced pathologies of internal organs correlated with elevated levels of alcohol biomarkers (GGT, PEth, EtG and EtS). Furthermore, GGT and PEth – biomarkers that show chronic or heavy drinking, had significant trend between their levels and number of pathology classes. At the same time, the levels of biomarkers of recent ethanol consumption – EtG and EtS, showed difference between cases with no organ-pathologies found versus those with any number of pathology present.

6.3. Coding problems of underlying cause of death in presence of multiple alcohol-related pathologies

During this study of premature death ICD-10 was used coding the cause of death. In Estonia forensic doctors usually write the diagnose on the death certificate and it is coded by an expert of the Estonian Causes of Death Registry. Diagnosing and coding deaths caused by multi-organ alcohol damage is challenging. Finding multiple serious competitive alcohol-related organ-pathologies at autopsy creates a problem of assigning one single underlying cause of death. A broad diagnosis of 'chronic end-organ damage' would be preferable in such cases and was used frequently by forensic doctors while filling death certificates during our study, but ICD-10 does not include such diagnose. Mentioning no single organ on death certificate led to situation where these deaths were assigned by the Statistical Office as having the underlying cause of F10.2 (Alcohol dependence syndrome). As it is controversial while F10.2 being a clinical psychiatric diagnosis is established *post mortem*, then forensic doctors try to choose the pathology of one organ as the underlying cause of death (mostly liver damage, K70) and add other alcohol-related diseases as accompanying diseases on the death certificate. Probably it has contributed to decrease of F10 and increase of K70 as alcohol-related causes of death during 2008-2019. Unfortunately, new ICD-11 that is going to be implemented in 2022 does not give the opportunity to have a combined diagnose of multiple organ-damage as single underlying cause of death.

According to literature, different BAC values are considered to be lethal – from 3.0 mg/g and over to 4.0 mg/g or more (Kugelberg and Jones, 2007; Lahti et al., 2011). In Estonia, it is recommended that BAC 3.0 mg/g or over should be present to diagnose acute alcohol poisoning (Drummer 2001), but with serious liver-damage, e.g. liver cirrhosis, acute poisoning can be diagnosed at lower BAC values (2.5 mg/g). At the same time, as alcohol-related deaths are usually considered stigmatizing, remains the possibility of their underreporting and mentioning more neutral causes of death on death certificates. Rahu and colleagues (Rahu et al., 2011) have also shown in their study significant decrease of reported alcohol poisonings (X45) and increase of mental disorders due to alcohol (F10) starting from 2000 in Estonia most possibly caused by misclassification of coding alcohol poisoning. In conclusion, the misclassification of acute alcohol poisonings can act in both directions.

Determining the cause of death in cases without evidence of acute alcohol toxicity or heart abnormalities but with just fatty liver present is not uncommon and presents a challenge. Firstly, alcoholic ketoacidosis as an immediate cause of death should be determined or excluded. Determining betahydroxybutyrate as a reliable marker of ketoacidosis in blood or humour vitreous is suggested by different authors (Klaric et al., 2020; Midtlyng et al., 2021). Moreover, the concentration of betahydroxybutyrate can indicate the origin of ketoacidosis, diabetic or alcoholic, especially in case of lacking preliminary medical data of the deceased. The capability of measuring betahydroxybutyrate is present at the

toxicology laboratory of Estonian Forensic Science Institute and this analysis should be used more often in the future. Secondly, Sorkin and colleagues (Sorkin & Sheppard, 2017) suggest that aforementioned cases of sudden death should be also differentiated from sudden arrhythmic death syndrome caused by hereditable channelopathies.

One more problem is to differentiate between alcoholic and non-alcoholic liver disease at autopsy. Non-alcoholic liver disease is defined by the presence of steatosis of more than 5% of hepatocytes with little or no alcohol consumption (Sanyal et al., 2015) and its risk factors are type II diabetes, insulin resistance, obesity and metabolic syndrome. Although, there are histological findings that indicate the type of liver disease (Takahashi & Fukusato, 2014), the most important diagnostic factor is the previous history of alcohol use. This confirms the importance of the available information from medical records of the deceased for the forensic doctor. During our study the medical records were frequently not available, but this is not the case anymore as currently the medical records can be reviewed.

6.4. Fatal poisonings caused by alcohol, psychotropic and other substances

During the study period of 2000–2009, 2.4% of all deaths in Estonia were caused by poisonings with ethanol being the most frequent cause of poisoning deaths followed by poisonings with carbon monoxide and illegal drugs. During this period, the decrease in alcohol poisonings and in last years in carbon monoxide poisonings was observed. The last one is probably related to the mandatory smoke detectors/fire alarm sensors since 2010 and carbon monoxide detectors since 2018 in the households in Estonia (Fire Safety Act, 2021). The decrease of alcohol poisonings has no one clear reason. One of the possible reasons being the change of law in 2006 that made it possible for family doctors to issue death certificates in case of suspected disease without autopsy and toxicological analyses (Establishment of Cause of Death Act, 2021).

During the years of 2000–2009 the mortality from illegal drug poisonings was 6.3 per 100 000 inhabitants in Estonia, being higher than in Finland but still lower than in Latvia according to EMCDDA reports. The sharp increases of poisoning deaths in 2002 and 2004 were remarkable, and were preceded by fentanyl and 3-methylfentanyl reaching Estonian market, respectively. Before that, in 2000–2002, poisonings with heroin and morphine were prevalent (Ojanperä et al., 2008). During the observed period majority (65.3%) of illegal drug poisonings were caused by fentanyles. In comparison with other countries (Carson et al., 2010), fatalities caused by misuse of fentanyl patches were not described in Estonia. During the study period no deaths by buprenorphine were determined, however approximately 6% of poisonings were caused by methadone. Both substances are used for substitution therapy and also illegally by drug addicts. In Estonia, methadone is more frequently used probably due to its

lower price, so this can explain why no poisonings by buprenorphine were detected.

In 2000–2009, of all poisonings approximately half were mono-intoxications involving mostly alcohol-related deaths. Poly-drug poisonings were frequent in poisonings with carbon monoxide and illegal drugs, illegal drugs were mainly combined with benzodiazepines and amphetamine. At the same time BAC was below 0.2 mg/g in 61% of illegal-drug deaths.

Determination of intention of death in fatal intoxications is challenging and is depending on preliminary data from police and the deceased person's health records. During the study most of the fatal poisonings were classified as accidents (92.7%) and in almost 4% of cases the intention remained undetermined. Assaults by poisoning were extremely rare.

In addition to study years 2000–2009, information of poisoning deaths was collected for next ten years, 2010–2019. Comparing these two decades, the total number of poisoning deaths decreased, and the whole number of autopsies performed at EFSI, as well, in 2010–2019. At the same time, the proportion of poisoning fatalities slightly increased from 14.3% of all autopsies in 2000–2009 to 17.6% in 2010–1019. There has been well noticeable decrease of poisonings with carbon monoxide – approximately two thirds less deaths caused by carbon monoxide were recorded in 2010–2019. In poisonings with illegal drugs there has been sharp decline in 2018–2019, probably caused by effective police work and also shutting down fentanyl labs.

6.5. Major strengths and limitations of the study

Strengths of the study

1. Study I, based on forensic autopsies of 25–54-years-old men, enabled a systematic in-depth collection of information about alcohol exposure provided by proxies, about the presence/absence of all pathologies, regardless of whether or not they were considered to have contributed to the death. At the same time, it went considerably beyond what would be recorded routinely at forensic autopsy.
2. Obtaining a fairly big sample of autopsies for the study enabled to analyse associations between alcohol exposure, organ damage and alcohol biomarkers.
3. Higher prevalence of heavy drinkers among deaths subjected to forensic autopsy together with alcohol exposure provided an excellent source of cases for understanding more about the presence of multiple alcohol-related pathologies as well as taking a close look at the principles of coding of underlying cause of death.
4. Study II, based on poisoning death among general population, described in detail all poisoning deaths over the 20 years (2000–2019) with main emphasis on alcohol and psychotropic substances poisonings.

Limitations of the study

1. The study was restricted to deaths subject to forensic autopsy. We did not have access to medical records and had no information on medical history of the study subjects at this time.
2. Brain pathology results would have been a valuable addition to our data, but was not included in the study because of lack of neuropathology expertise in Estonia.
3. Restricted information on alcohol exposure regarding sample size and accuracy of information. We could only reach families of those men who had lived in the five biggest cities. Also, the only source of alcohol exposure information was proxies who may provide less reliable information on alcohol exposure and this information may be biased due to recall and social responsibility.
4. Toxicology was based on preliminary data as in routine forensic practice no predefined list of medicine for the toxicology analysis exists.

7. CONCLUSIONS

This study gave an evidence-based overview of the alcohol exposure, underlying causes of death, existence and the extent of pathologies and alcohol biomarkers detected at forensic autopsy among working-age men and of fatal poisonings caused by alcohol, psychotropic and other substances among general population over two decades in Estonia.

1. Alcohol-related pathologies are common among working age men subject to forensic autopsy in Estonia indicating harmful alcohol consumption in this age group.
2. Several of the novel alcohol biomarkers (Etg, Ets and PEth) appear to perform well in *post mortem* samples.
3. In case of multiple alcohol-related pathologies it is not possible to allocate underlying cause of death because of rigidity of classification.
4. Among poisoning deaths in Estonia, ethanol was the most frequent cause of death in 2000–2009, but psychotropic substances prevailed in 2010–2019.

8. MAIN PRACTICAL IMPLICATIONS

The findings of this study are useful for health policy makers and institutions involved in forensic and patoanatomical autopsies in Estonia.

More specific suggestions are the following:

1. To use *post mortem* alcohol biomarkers (Etg, Ets and PEth) more widely to identify both recent and chronic alcohol use.
2. To develop and incorporate neuropathology into forensic practice in order to obtain more complete information concerning alcohol-related organ-pathology.
3. To advise using the leading organ-pathology as the underlying cause of death on the death certificate in case of multiple organ-damage by alcohol.
4. To increase the use of betahydroxybutyrate as an indicator of significant ketoacidosis as the possible direct cause of death in case of alcohol-related harm.
5. To improve the quality of forensic database concerning poisoning deaths by regular quality checks and routine validation against autopsy protocols.

9. REFERENCES

Akechi, T., Iwasaki, M., Uchitomi, Y., & Tsugane, S. (2006). Alcohol consumption and suicide among middle-aged men in Japan. *The British Journal of Psychiatry*, *188*, 231–236. https://doi.org/10.1192/bjp.188.3.231

Andresen-Streichert, H., Müller, A., Glahn, A., Skopp, G., & Sterneck, M. (2018). Alcohol biomarkers in clinical and forensic contexts. *Deutsches Arzteblatt International*, *115*, 309–315. https://doi.org/10.3238/arztebl.2018.0309

Anttila, P., Järvi, K., Latvala, J., Romppanen, J., Punnonen, K., & Niemelä, O. (2005). Biomarkers of alcohol consumption in patients classified according to the degree of liver disease severity. *Scandinavian Journal of Clinical and Laboratory Investigation*, *65*, 141–151. https://doi.org/10.1080/00365510510013532

Arakawa, M., Masuzaki, T., & Okuda, K. (2002). Pathomorphology of esophageal and gastric varices. *Seminars in Liver Disease*, *22*, 73–82. https://doi.org/10.1055/s-2002-23208

Arvaniti, V., D'Amico, G., Fede, G., Manousou, P., Tsochatzis, E., Pleguezuelo, M., & Burroughs, A. K. (2010). Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. *Gastroenterology*, *139*, 1246–1256, 1256.e1-5. https://doi.org/10.1053/j.gastro.2010.06.019

Baburin, A., Reile, R., Veideman, T., & Leinsalu, M. (2020). Age, period and cohort effects on alcohol consumption in Estonia, 1996–2018. *Alcohol and Alcoholis*m, 56, 451–459. https://doi.org/10.1093/alcalc/agaa115

Bernard, J.-P., Havnes, I., Slørdal, L., Waal, H., Mørland, J., & Khiabani, H. Z. (2013). Methadone-related deaths in Norway. *Forensic Science International*, *224*, 111–116. https://doi.org/10.1016/j.forsciint.2012.11.010

Bishehsari, F., Magno, E., Swanson, G., Desai, V., Voigt, R. M., Forsyth, C. B., & Keshavarzian, A. (2017). Alcohol and gut-derived inflammation. *Alcohol Research : Current Reviews*, *38*, 163–171.

Bogstrand, S. T., Normann, P. T., Rossow, I., Larsen, M., Mørland, J., & Ekeberg, Ø. (2011). Prevalence of alcohol and other substances of abuse among injured patients in a Norwegian emergency department. *Drug and Alcohol Dependence*, *117*, 132–138. https://doi.org/10.1016/j.drugalcdep.2011.01.007

Britton, A., & McKee, M. (2000). The relation between alcohol and cardiovascular disease in Eastern Europe: Explaining the paradox. *Journal of Epidemiology and Community Health*, *54*, 328–332. https://doi.org/10.1136/jech.54.5.328

Bujanda, L. (2000). The effects of alcohol consumption upon the gastrointestinal tract. *The American Journal of Gastroenterology*, *95*, 3374–3382. https://doi.org/10.1111/j.1572-0241.2000.03347.x

Butterworth, R. F. (2000). Complications of cirrhosis III. Hepatic encephalopathy. *Journal of Hepatology*, *32(Suppl)*, 171–180. https://doi.org/10.1016/s0168-8278(00)80424-9

Carson, H. J., Knight, L. D., Dudley, M. H., & Garg, U. (2010). A fatality involving an unusual route of fentanyl delivery: Chewing and aspirating the transdermal patch. *Legal Medicine*, *12*, 157–159. https://doi.org/10.1016/j.legalmed.2010.03.001

Cederbaum, A. I. (2012). Alcohol metabolism. *Clinics in Liver Disease*, *16*, 667–685. https://doi.org/10.1016/j.cld.2012.08.002

Chacko, K. R., & Reinus, J. (2016). Spectrum of alcoholic liver disease. *Clinics in Liver Disease*, *20*(3), 419–427. https://doi.org/10.1016/j.cld.2016.02.002

Clemens, D. L., & Mahan, K. J. (2010). Alcoholic pancreatitis: lessons from the liver. *World Journal of Gastroenterology*, *16*, 1314–1320. https://doi.org/10.3748/wjg.v16.i11.1314

Conwell, D. L., Banks, P. A., Sandhu, B. S., Sherman, S., Al-Kaade, S., Gardner, T. B., Anderson, M. A., Wilcox, C. M., Lewis, M. D., Muniraj, T., Forsmark, C. E., Cote, G. A., Guda, N. M., Tian, Y., Romagnuolo, J., Wisniewski, S. R., Brand, R., Gelrud, A., Slivka, A., … Yadav, D. (2017). Validation of demographics, etiology, and risk factors for chronic pancreatitis in the USA: A report of the North American Pancreas Study (NAPS) Group. *Digestive Diseases and Sciences*, *62*, 2133–2140. https://doi.org/10.1007/s10620-017-4621-z

Conwell, D. L., Lee, L. S., Yadav, D., Longnecker, D. S., Miller, F. H., Mortele, K. J., Levy, M. J., Kwon, R., Lieb, J. G., Stevens, T., Toskes, P. P., Gardner, T. B., Gelrud, A., Wu, B. U., Forsmark, C. E., & Vege, S. S. (2014). American Pancreatic Association Practice Guidelines in Chronic Pancreatitis: evidence-based report on diagnostic guidelines. *Pancreas*, *43*, 1143–1162. https://doi.org/10.1097/MPA.0000000000000237

Cotran, R., Kumar, V., Collins, T., & Robbins, S. (1999). *Robbins pathologic basis of disease* (6th edition). Philadelphia, Saunders.

Danilova, I., Shkolnikov, V. M., Andreev, E., & Leon, D. A. (2020). *The changing relation between alcohol and life expectancy in Russia in 1965 – 2017*. *Drug and Alcohol Review, 39,* 790–796. https://doi.org/10.1111/dar.13034

Day, E., & Rudd, J. H. F. (2019). Alcohol use disorders and the heart. *Addiction*, *114*, 1670–1678. https://doi.org/10.1111/add.14703

de la Monte, S. M., & Kril, J. J. (2014). Human alcohol-related neuropathology. *Acta Neuropathologica*, *127*, 71–90. https://doi.org/10.1007/s00401-013-1233-3

Dowell, D., Noonan, R. K., & Houry, D. (2017). Underlying factors in drug overdose deaths. *JAMA, 318,* 2295–2296. https://doi.org/10.1001/jama.2017.15971

Drummer, O. H. (2019). Fatalities caused by novel opioids: a review. In *Forensic Sciences Research, 4*, 95–110. https://doi.org/10.1080/20961790.2018.1460063

Drummer, O. H., & Odell, M. (2001). *The forensic pharmacology of drugs of abuse* (1st edition). London, Arnold.

Engen, P. A., Green, S. J., Voigt, R. M., Forsyth, C. B., & Keshavarzian, A. (2015). The gastrointestinal microbiome: alcohol effects on the composition of intestinal microbiota. *Alcohol Research: Current Reviews*, *37*, 223–236.

Establishment of Cause of Death Act. (2021). Riigi Teataja. https://www.riigiteataja.ee/en/eli/ee/525062018018/consolide/current

Estonian Forensic Science Institute. (2021). https://www.ekei.ee/en/

Estonian Institute of Economic Research. (2020). *Alcohol market, consumption and harms in Estonia Yearbook 2020.* https://intra.tai.ee/images/prints/documents/160561083224_Alkoholi_turg_tarbimine_2020.pdf

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). (2020). *European Drug Report 2020: Trends and Developments*. https://www.emcdda.europa.eu/system/files/publications/13236/TDAT20001ENN_web.pdf

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). (2019). *Drug-related deaths and mortality in Europe*. https://www.emcdda.europa.eu/system/files/publications/11485/20193286_TD0319444ENN_PDF.pdf

Fire Safety Act. (2021) Riigi Teataja. https://www.riigiteataja.ee/en/eli/ee/529032021004/consolide/current

Flanagan, R. J. (2008). Fatal toxicity of drugs used in psychiatry. *Human Psychopharmacology*, *23 Suppl 1*, 43–51. https://doi.org/10.1002/hup.916

Forensic Examination Act. (2021). Riigi Teataja. https://www.riigiteataja.ee/en/eli/ee/530102013102/consolide/current

Franke, A., Teyssen, S., & Singer, M. V. (2005). Alcohol-related diseases of the esophagus and stomach. *Digestive Diseases, 23*, 204–213. https://doi.org/10.1159/000090167

Frisoni, P., Bacchio, E., Bilel, S., Talarico, A., Gaudio, R. M., Barbieri, M., Neri, M., & Marti, M. (2018). Novel synthetic opioids: The pathologist's point of view. *Brain Sciences*, 8, 170. https://doi.org/10.3390/brainsci8090170

Fumeaux, L., Scarpelli, M. P., Tettamanti, C., & Palmiere, C. (2018). Usefulness of liver function tests in postmortem samples. *Journal of Forensic and Legal Medicine*, *56*, 51–54. https://doi.org/10.1016/j.jflm.2018.03.011

Gallagher, C., Hendriks, J. M. L., Elliott, A. D., Wong, C. X., Rangnekar, G., Middeldorp, M. E., Mahajan, R., Lau, D. H., & Sanders, P. (2017). Alcohol and incident atrial fibrillation – A systematic review and meta-analysis. *International Journal of Cardiology*, *246*, 46–52. https://doi.org/10.1016/j.ijcard.2017.05.133

Gamble, L., Mason, C. M., & Nelson, S. (2006). The effects of alcohol on immunity and bacterial infection in the lung. *Medecine et Maladies Infectieuses*, *36*, 72–77. https://doi.org/10.1016/j.medmal.2005.08.010

Gao, B., & Bataller, R. (2011). Alcoholic liver disease: pathogenesis and new therapeutic targets. *Gastroenterology*, *141*, 1572–1585. https://doi.org/10.1053/j.gastro.2011.09.002

George, A., & Figueredo, V. M. (2011). Alcoholic cardiomyopathy: a review. *Journal of Cardiac Failure*, *17*, 844–849. https://doi.org/10.1016/j.cardfail.2011.05.008

Gummin, D. D., Mowry, J. B., Beuhler, M. C., Spyker, D. A., Brooks, D. E., Dibert, K. W., Rivers, L. J., Pham, N. P. T., & Ryan, M. L. (2020). 2019 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 37th Annual Report. *Clinical Toxicology*, *58*), 1360–1541. https://doi.org/10.1080/15563650.2020.1834219

Guzzo-Merello, G., Cobo-Marcos, M., Gallego-Delgado, M., & Garcia-Pavia, P. (2014). Alcoholic cardiomyopathy. *World Journal of Cardiology*, *6*, 771–781. https://doi.org/10.4330/wjc.v6.i8.771

Hagström, H. (2017). Alcohol consumption in concomitant liver disease: How much is too much? *Current Hepatology Reports*, *16*, 152–157. https://doi.org/10.1007/s11901-017-0343-0

Handley, S., Patel, M. X., & Flanagan, R. J. (2016). Antipsychotic-related fatal poisoning, England and Wales, 1993–2013: impact of the withdrawal of thioridazine. *Clinical Toxicology, 54*, 471–480. https://doi.org/10.3109/15563650.2016.1164861

Harper, C, & Corbett, D. (1990). Changes in the basal dendrites of cortical pyramidal cells from alcoholic patients – a quantitative Golgi study. *Journal of Neurology, Neurosurgery, and Psychiatry*, *53*, 856–861. https://doi.org/10.1136/jnnp.53.10.856

Harper, Clive, & Matsumoto, I. (2005). Ethanol and brain damage. *Current Opinion in Pharmacology*, *5*, 73–78. https://doi.org/10.1016/j.coph.2004.06.011

Hart, P. A., & Conwell, D. L. (2020). Chronic pancreatitis: Managing a difficult disease. *The American Journal of Gastroenterology*, *115*, 49–55. https://doi.org/10.14309/ajg.0000000000000421

Health Statistics and Health Research Database. (2021). National Institute for Health Development. https://statistika.tai.ee/index_en.html

Helander, A. (2003). Biological markers in alcoholism. *Journal of Neural Transmission. Supplementum*, *66*, 15–32. https://doi.org/10.1007/978-3-7091-0541-2_2

Helander, A., & Dahl, H. (2005). Urinary tract infection: a risk factor for false-negative urinary ethyl glucuronide but not ethyl sulfate in the detection of recent alcohol consumption. *Clinical Chemistry*, *51*, 1728–1730. https://doi.org/10.1373/clinchem.2005.051565

Helander, A., Olsson, I., & Dahl, H. (2007). Postcollection synthesis of ethyl glucuronide by bacteria in urine may cause false identification of alcohol consumption. *Clinical Chemistry*, *53*, 1855–1857. https://doi.org/10.1373/clinchem.2007.089482

Helander, A., Wielders, J., Anton, R., Arndt, T., Bianchi, V., Deenmamode, J., Jeppsson, J.-O., Whitfield, J. B., Weykamp, C., & Schellenberg, F. (2016). Standardisation and use of the alcohol biomarker carbohydrate-deficient transferrin (CDT). *Clinica Chimica Acta*, *459*, 19–24. https://doi.org/10.1016/j.cca.2016.05.016

Hietanen, S., Herajärvi, J., Junttila, J., Pakanen, L., Huikuri, H. V, & Liisanantti, J. (2020). Characteristics of subjects with alcoholic cardiomyopathy and sudden cardiac death. *Heart*, *106*, 686–690. https://doi.org/10.1136/heartjnl-2019-315534

Hovda, K. E., Hunderi, O. H., Tafjord, A. B., Dunlop, O., Rudberg, N., & Jacobsen, D. (2005). Methanol outbreak in Norway 2002–2004: Epidemiology, clinical features and prognostic signs. In *Journal of Internal Medicine, 258,* 181–190. https://doi.org/10.1111/j.1365-2796.2005.01521.x

Irving, H. M., Samokhvalov, A. V, & Rehm, J. (2009). Alcohol as a risk factor for pancreatitis. A systematic review and meta-analysis. *Journal of the Pancreas*, *10*, 387–392.

Isaksson, A., Walther, L., Hansson, T., Andersson, A., & Alling, C. (2011). Phosphatidylethanol in blood (B-PEth): a marker for alcohol use and abuse. *Drug Testing and Analysis*, *3*, 195–200. https://doi.org/10.1002/dta.278

Janík, M., Ublová, M., Kučerová, Š., & Hejna, P. (2017). Carbon monoxide-related fatalities: A 60-year single institution experience. *Journal of Forensic and Legal Medicine*, *48*, 23–29. https://doi.org/10.1016/j.jflm.2017.04.002

Jasilionis, D., Leon, D. A., & Pechholdová, M. (2020). Impact of alcohol on mortality in Eastern Europe: Trends and policy responses. *Drug and Alcohol Review*, *39*, 785–789. https://doi.org/10.1111/dar.13167

Jastrzębska, I., Zwolak, A., Szczyrek, M., Wawryniuk, A., Skrzydło-Radomańska, B., & Daniluk, J. (2016). Biomarkers of alcohol misuse: recent advances and future prospects. *Przeglad Gastroenterologiczny*, *11*, 78–89. https://doi.org/10.5114/pg.2016.60252

Jones, A. W., Kugelberg, F. C., Holmgren, A., & Ahlner, J. (2011). Drug poisoning deaths in Sweden show a predominance of ethanol in mono-intoxications, adverse drug-alcohol interactions and poly-drug use. In *Forensic Science International, 206*, 43–51. https://doi.org/10.1016/j.forsciint.2010.06.015

Jonsson, A., Bonander, C., Nilson, F., & Huss, F. (2017). The state of the residential fire fatality problem in Sweden: Epidemiology, risk factors, and event typologies. *Journal of Safety Research*, *62*, 89–100. https://doi.org/10.1016/j.jsr.2017.06.008

Kaphalia, L., & Calhoun, W. J. (2013). Alcoholic lung injury: metabolic, biochemical and immunological aspects. *Toxicology Letters*, *222*, 171–179. https://doi.org/10.1016/j.toxlet.2013.07.016

Kershaw, C. D., & Guidot, D. M. (2008). Alcoholic lung disease. *Alcohol Research & Health, 31*, 66–75.

Klaric, K.-A., Milroy, C. M., & Parai, J. L. (2020). Utility of postmortem vitreous beta-hydroxybutyrate testing for distinguishing sudden from prolonged deaths and for diagnosing ketoacidosis. *Journal of Forensic Sciences*, *65*, 1588–1593. https://doi.org/10.1111/1556-4029.14443

Klatsky, A. L. (2010). Alcohol and cardiovascular health. *Physiology & Behavior*, *100*, 76–81. https://doi.org/10.1016/j.physbeh.2009.12.019

Knoll, M. R., Kölbel, C. B., Teyssen, S., & Singer, M. V. (1998). Action of pure ethanol and some alcoholic beverages on the gastric mucosa in healthy humans: a descriptive endoscopic study. *Endoscopy*, *30*, 293–301. https://doi.org/10.1055/s-2007-1001257

Krabseth, H., Mørland, J., & Høiseth, G. (2014). Assistance of ethyl glucuronide and ethyl sulfate in the interpretation of postmortem ethanol findings. *International Journal of Legal Medicine*, *128*, 765–770. https://doi.org/10.1007/s00414-014-1031-z

Kriikku, P., Häkkinen, M., & Ojanperä, I. (2018). High buprenorphine-related mortality is persistent in Finland. *Forensic Science International*, *291*, 76–82. https://doi.org/10.1016/j.forsciint.2018.08.010

Kugelberg, F. C., & Jones, A. W. (2007). Interpreting results of ethanol analysis in postmortem specimens: a review of the literature. *Forensic Science International*, *165*, 10–29. https://doi.org/10.1016/j.forsciint.2006.05.004

Lahti, R. A., Sajantila, A., Korpi, H., Poikolainen, K., & Vuori, E. (2011). Under-recording of ethanol intoxication and poisoning in cause-of-death data: causes and consequences. *Forensic Science International*, *212*, 121–125. https://doi.org/10.1016/j.forsciint.2011.05.029

Lahti, R. A., Sajantila, A., Korpi, H., Poikolainen, K., & Vuori, E. (2011). Under-recording of ethanol intoxication and poisoning in cause-of-death data: Causes and consequences. *Forensic Science International, 212*, 121–125. https://doi.org/10.1016/j.forsciint.2011.05.029

Lefrancois, E., Reymond, N., Thomas, A., Lardi, C., Fracasso, T., & Augsburger, M. (2021). Summary statistics for drugs and alcohol concentration recovered in post-mortem femoral blood in Western Switzerland. *Forensic Science International*, *325*, 110883. https://doi.org/10.1016/j.forsciint.2021.110883

Leon, D. A., Chenet, L., Shkolnikov, V. M., Zakharov, S., Shapiro, J., Rakhmanova, G., Vassin, S., & McKee, M. (1997). Huge variation in Russian mortality rates 1984–94: artefact, alcohol, or what? *The Lancet*, *350*, 383–388. https://doi.org/10.1016/S0140-6736(97)03360-6

Leon, D. A., Saburova, L., Tomkins, S., Andreev, E., Kiryanov, N., Mckee, M., & Shkolnikov, V. M. (2007). Hazardous alcohol drinking and premature mortality in Russia: a population based case-control study. *Lancet, 369*, 2001–2009. https://doi.org/10.1016/S0140-6736(07)60941-6

Leon, D. A., Shkolnikov, V., McKee, M., Kiryanov, N., & Andreev, E. (2010). Alcohol increases circulatory disease mortality in Russia: Acute and chronic effects or misattribution of cause? *International Journal of Epidemiology*, *39*, 1279–1290. https://doi.org/10.1093/ije/dyq102

Lesmana, C. R. A., Raharjo, M., & Gani, R. A. (2020). Managing liver cirrhotic complications: Overview of esophageal and gastric varices. *Clinical and Molecular Hepatology*, *26*, 444–460. https://doi.org/10.3350/cmh.2020.0022

Levine, M., & Ruha, A.-M. (2012). Overdose of atypical antipsychotics: clinical presentation, mechanisms of toxicity and management. *CNS Drugs*, *26*, 601–611. https://doi.org/10.2165/11631640-000000000-00000

Mackenbach, J. P., Kulhánová, I., Bopp, M., Borrell, C., Deboosere, P., Kovács, K., Looman, C. W. N., Leinsalu, M., Mäkelä, P., Martikainen, P., Menvielle, G., Rodríguez-Sanz, M., Rychtaříková, J., & de Gelder, R. (2015). Inequalities in alcohol-related mortality in 17 European countries: A retrospective analysis of mortality registers. *PLoS Medicine, 12.* https://doi.org/10.1371/journal.pmed.1001909

Mäkelä, P. (1998). Alcohol-related mortality by age and sex and its impact on life expectancy. *The European Journal of Public Health*, *8*, 43–51. https://doi.org/10.1093/eurpub/8.1.43

McCartney, G., Mahmood, L., Leyland, A. H., Batty, G. D., & Hunt, K. (2011). Contribution of smoking-related and alcohol-related deaths to the gender gap in mortality: evidence from 30 European countries. *Tobacco Control*, *20*, 166–168. https://doi.org/10.1136/tc.2010.037929

McKee, M., & Shkolnikov, V. (2001). Understanding the toll of premature death among men in eastern Europe. *BMJ*, *323*, 1051–1055. https://doi.org/10.1136/bmj.323.7320.1051

Michel, L., Serrano, A., & Malt, R. A. (1980). Mallory-Weiss syndrome. Evolution of diagnostic and therapeutic patterns over two decades. *Annals of Surgery*, *192*, 716–721. https://doi.org/10.1097/00000658-198012000-00004

Midtlyng, L., Høiseth, G., Luytkis, H., Kristoffersen, L., Le Nygaard, I., Strand, M. C., Arnestad, M., & Vevelstad, M. (2021). Relationship between betahydroxybutyrate (BHB) and acetone concentrations in postmortem blood and cause of death. *Forensic Science International*, *321*, 110726. https://doi.org/10.1016/j.forsciint.2021.110726

Mirijello, A., Tarli, C., Vassallo, G. A., Sestito, L., Antonelli, M., d'Angelo, C., Ferrulli, A., De Cosmo, S., Gasbarrini, A., & Addolorato, G. (2017). Alcoholic cardiomyopathy: What is known and what is not known. *European Journal of Internal Medicine, 43*, pp. 1–5. https://doi.org/10.1016/j.ejim.2017.06.014

Mounteney, J., Giraudon, I., Denissov, G., & Griffiths, P. (2015). Fentanyls: Are we missing the signs? Highly potent and on the rise in Europe. *International Journal of Drug Policy, 26*, 626–631. https://doi.org/10.1016/j.drugpo.2015.04.003

Musshoff, F. (2002). Chromatographic methods for the determination of markers of chronic and acute alcohol consumption. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, *781*, 457–480. https://doi.org/10.1016/s1570-0232(02)00691-8

Nanau, R. M., & Neuman, M. G. (2015). Biomolecules and biomarkers used in diagnosis of alcohol drinking and in monitoring therapeutic interventions. *Biomolecules*, *5*, 1339–1385. https://doi.org/10.3390/biom5031339

Navasa, M., Rimola, A., & Rodés, J. (1997). Bacterial infections in liver disease. *Seminars in Liver Disease*, *17*, 323–333. https://doi.org/10.1055/s-2007-1007209

Neumann, J., Becdk, O., Helander, A., & Böttcher, M. (2020). Performance of PEth compared with other alcohol biomarkers in subjects presenting for occupational and pre-employment medical examination. *Alcohol and Alcoholism*, *55*, 401–408. https://doi.org/10.1093/alcalc/agaa027

Niemelä, O. (2016). Biomarker-based approaches for assessing alcohol use disorders. *International Journal of Environmental Research and Public Health*, *13*, 166. https://doi.org/10.3390/ijerph13020166

Nivukoski, U., Bloigu, A., Bloigu, R., Aalto, M., Laatikainen, T., & Niemelä, O. (2019). Liver enzymes in alcohol consumers with or without binge drinking. *Alcohol, 78*, 13–19. https://doi.org/10.1016/j.alcohol.2019.03.001

Noble, J. M., & Weimer, L. H. (2014). Neurologic complications of alcoholism. *Neurology of Systemic Disease, 20*, 624–641. https://doi.org/10.1212/01.CON.0000450970.99322.84

Nordrum, I., Eide, T. J., & Jørgensen, L. (2000). Alcohol in a series of medico-legally autopsied deaths in northern Norway 1973–1992. *Forensic Science International*, *110*, 127–137. https://doi.org/10.1016/S0379-0738(00)00163-8

Ojanperä, I., Gergov, M., Liiv, M., Riikoja, A., & Vuori, E. (2008). An epidemic of fatal 3-methylfentanyl poisoning in Estonia. *International Journal of Legal Medicine, 122*, 395–400. https://doi.org/10.1007/s00414-008-0230-x

Paasma, R., Hovda, K. E., Tikkerberi, A., & Jacobsen, D. (2007). Methanol mass poisoning in Estonia: Outbreak in 154 patients. *Clinical Toxicology,* 45, 152–157. https://doi.org/10.1080/15563650600956329

Pärna, K. (2020). Alcohol consumption and alcohol policy in Estonia 2000–2017 in the context of Baltic and Nordic countries. *Drug and Alcohol Review,* 39, 797–804). https://doi.org/10.1111/dar.13008

Pärna, K., & Leon, D. A. (2011). Surrogate alcohol drinking in Estonia. *Alcoholism: Clinical and Experimental Research*, *35*, 1454–1457. https://doi.org/10.1111/j.1530-0277.2011.01481.x

Pärna, K., & Rahu, K. (2010). Dramatic increase in alcoholic liver cirrhosis mortality in Estonia in 1992–2008. *Alcohol and Alcoholism*, *45*, 548–551. https://doi.org/-10.1093/alcalc/agq050

Pärna, K., Rahu, K., Helakorpi, S., & Tekkel, M. (2010). Alcohol consumption in Estonia and Finland: Finbalt survey 1994–2006. *BMC Public Health*, *10*, 261. https://doi.org/10.1186/1471-2458-10-261

Persson, E., Schwartz, L., Park, Y., Trabert, B., Hollenbeck, A., Graubard, B., Freedman, N., & McGlynn, K. (2013). Alcohol consumption, folate intake, hepatocellular carcinoma, and liver disease mortality. *Cancer Epidemiology, Biomarkers and Prevention*, *22*, 415–421. https://doi.org/10.1158/1055-9965.EPI-12-1169

Philips, C. A., Augustine, P., Yerol, P. K., Rajesh, S., & Mahadevan, P. (2019). Severe alcoholic hepatitis: current perspectives. *Hepatic Medicine: Evidence and Research*, *11*, 97–108. https://doi.org/10.2147/HMER.S197933

Piano, M. R., & Phillips, S. A. (2014). Alcoholic cardiomyopathy: pathophysiologic insights. *Cardiovascular Toxicology*, *14*, 291–308. https://doi.org/10.1007/s12012-014-9252-4

Pomerleau, J., McKee, M., Rose, R., Haerpfer, C. W., Rotman, D., & Tumanov, S. (2008). Hazardous alcohol drinking in the former Soviet Union: a cross-sectional study of eight countries. *Alcohol and Alcoholism*, *43*, 351–359. https://doi.org/10.1093/alcalc/agm167

Popova, S., Rehm, J., Patra, J., & Zatonski, W. (2007). Comparing alcohol consumption in central and eastern Europe to other European countries. *Alcohol and Alcoholism, 42*, 465–473. https://doi.org/10.1093/alcalc/agl124

Prahlow, J. A., & Landrum, J. E. (2005). Amitriptyline abuse and misuse. *The American Journal of Forensic Medicine and Pathology*, *26*, 86–88. https://doi.org/10.1097/01.paf.0000154111.69255.ab

Pratt, D. S., & Kaplan, M. M. (2000). Evaluation of abnormal liver-enzyme results in asymptomatic patients. *The New England Journal of Medicine*, *342*, 1266–1271. https://doi.org/10.1056/NEJM200004273421707

Pruckner, N., Hinterbuchinger, B., Fellinger, M., König, D., Waldhoer, T., Lesch, O. M., Gmeiner, A., Vyssoki, S., & Vyssoki, B. (2019). Alcohol-related mortality in the WHO European Region: Sex-specific trends and predictions. *Alcohol and Alcoholism*, *54*, 593–598. https://doi.org/10.1093/alcalc/agz063

Rahu, K., Palo, E., & Rahu, M. (2011). Diminishing trend in alcohol poisoning mortality in estonia: reality or coding peculiarity? *Alcohol and Alcoholism, 46*, 485–489. https://doi.org/10.1093/alcalc/agr046

Rahu, K., Rahu, M., & Zeeb, H. (2019). Sex disparities in premature adult mortality in Estonia 1995–2016: a national register-based study. *BMJ Open*, *9*, e026210. https://doi.org/10.1136/bmjopen-2018-026210

Rainio, J., Ahola, S., Kangastupa, P., Kultti, J., Tuomi, H., Karhunen, P. J., Helander, A., & Niemelä, O. (2014). Comparison of ethyl glucuronide and carbohydrate-deficient transferrin in different body fluids for post-mortem identification of alcohol use. *Alcohol and Alcoholism, 49*, 55–59. https://doi.org/10.1093/alcalc/agt159

Rainio, J., de Giorgio, F., Bortolotti, F., & Tagliaro, F. (2008). Objective post-mortem diagnosis of chronic alcohol abuse--a review of studies on new markers. *Legal Medicine, 10*, 229–235. https://doi.org/10.1016/j.legalmed.2008.01.006

Rehm, J., Hasan, O. S. M., Imtiaz, S., & Neufeld, M. (2017). Quantifying the contribution of alcohol to cardiomyopathy: A systematic review. *Alcohol, 61*, 9–15. https://doi.org/10.1016/j.alcohol.2017.01.011

Rehm, J., Manthey, J., Shield, K. D., & Ferreira-Borges, C. (2019). Trends in substance use and in the attributable burden of disease and mortality in the WHO European Region, 2010–16. *European Journal of Public Health*, *29*, 723–728. https://doi.org/10.1093/eurpub/ckz064

Ricci, C., Wood, A., Muller, D., Gunter, M. J., Agudo, A., Boeing, H., van der Schouw, Y. T., Warnakula, S., Saieva, C., Spijkerman, A., Sluijs, I., Tjønneland, A., Kyrø, C., Weiderpass, E., Kühn, T., Kaaks, R., Sánchez, M.-J., Panico, S., Agnoli, C., … Ferrari, P. (2018). Alcohol intake in relation to non-fatal and fatal coronary heart disease and stroke: EPIC-CVD case-cohort study. *BMJ, 361*, k934. https://doi.org/10.1136/bmj.k934

Rocco, A., Compare, D., Angrisani, D., Sanduzzi Zamparelli, M., & Nardone, G. (2014). Alcoholic disease: liver and beyond. *World Journal of Gastroenterology*, *20*, 14652–14659. https://doi.org/10.3748/wjg.v20.i40.14652

Ruas, F., Mendonça, M. C., Real, F. C., Vieira, D. N., & Teixeira, H. M. (2014). Carbon monoxide poisoning as a cause of death and differential diagnosis in the forensic practice: A retrospective study, 2000–2010. *Journal of Forensic and Legal Medicine, 24*, 1–6. https://doi.org/10.1016/j.jflm.2014.02.002

Samokhvalov, A. V, Irving, H. M., & Rehm, J. (2010). Alcohol consumption as a risk factor for pneumonia: a systematic review and meta-analysis. *Epidemiology and Infection*, *138*, 1789–1795. https://doi.org/10.1017/S0950268810000774

Sanyal, A. J., Friedman, S. L., McCullough, A. J., & Dimick-Santos, L. (2015). Challenges and opportunities in drug and biomarker development for nonalcoholic steatohepatitis: findings and recommendations from an American Association for the Study of Liver Diseases – U.S. Food and Drug Administration Joint Workshop. *Hepatology, 61*, 1392–1405. https://doi.org/10.1002/hep.27678

Schulze, R. J., & Ding, W. X. (2019). Lipid droplet dynamics in alcoholic fatty liver disease. *Liver Research, 3*, 185–190. https://doi.org/10.1016/j.livres.2019.09.002

Schuppan, D., & Afdhal, N. H. (2008). Liver cirrhosis. *Lancet, 371*, 838–851. https://doi.org/10.1016/S0140-6736(08)60383-9

Seitz, H. K., Bataller, R., Cortez-Pinto, H., Gao, B., Gual, A., Lackner, C., Mathurin, P., Mueller, S., Szabo, G., & Tsukamoto, H. (2018). Alcoholic liver disease. *Nature Reviews. Disease Primers*, *4*, 16. https://doi.org/10.1038/s41572-018-0014-7

Shield, K. D., & Rehm, J. (2015). Global risk factor rankings : the importance of age - based health loss inequities caused by alcohol and other risk factors. *BMC Research Notes*, *8*, 231. https://doi.org/10.1186/s13104-015-1207-8

Simonsen, K. W., Christoffersen, D. J., Linnet, K., & Andersen, C. U. (2020). Fatal poisoning among drug users in Denmark in 2017. *Danish Medical Journal*, *68,* A07200560.

Singal, A. K., Bataller, R., Ahn, J., Kamath, P. S., & Shah, V. H. (2018). ACG Clinical Guideline: Alcoholic liver disease. *The American Journal of Gastroenterology*, *113*, 175–194. https://doi.org/10.1038/ajg.2017.469

Singhvi, A., Abromitis, R., Althouse, A. D., Bataller, R., Arteel, G. E., & Yadav, D. (2020). Coexistence of alcohol-related pancreatitis and alcohol-related liver disease: A systematic review and meta-analysis. *Pancreatology, 20*, 1069–1077. https://doi.org/10.1016/j.pan.2020.07.412

Sinha, S., Kataria, A., Kolla, B. P., Thusius, N., & Loukianova, L. L. (2019). Wernicke encephalopathy – clinical pearls. *Mayo Clinic Proceedings*, *94*, 1065–1072. https://doi.org/10.1016/j.mayocp.2019.02.018

Sjögren, H., Eriksson, A., & Ahlm, K. (2000). Alcohol and unnatural deaths in Sweden: a medico-legal autopsy study. *Journal of Studies on Alcohol*, *61*, 507–514. https://doi.org/10.15288/jsa.2000.61.507.

Smollin, C., & Olson, K. (2010). Carbon monoxide poisoning (acute). *BMJ Clinical Evidence*, *2010*.

Sorkin, T., & Sheppard, M. N. (2017). Sudden unexplained death in alcohol misuse (SUDAM) patients have different characteristics to those who died from sudden arrhythmic death syndrome (SADS). *Forensic Science, Medicine, and Pathology, 13*, 278–283. https://doi.org/10.1007/s12024-017-9877-2

Statistics Estonia. (2021). *Statistics Estonia*. https://www.stat.ee/en

Stickel, F., Datz, C., Hampe, J., & Bataller, R. (2017). Pathophysiology and management of alcoholic liver disease: Update 2016. *Gut and Liver*, *11*, 173–188. https://doi.org/10.5009/gnl16477

Stockings, E., Tran, L. T., Santo, T. J., Peacock, A., Larney, S., Santomauro, D., Farrell, M., & Degenhardt, L. (2019). Mortality among people with regular or problematic use of amphetamines: a systematic review and meta-analysis. *Addiction, 114*, 1738–1750. https://doi.org/10.1111/add.14706

Suzuki, A., Lymp, J., St Sauver, J., Angulo, P., & Lindor, K. (2006). Values and limitations of serum aminotransferases in clinical trials of nonalcoholic steatohepatitis. *Liver International*, *26*, 1209–1216. https://doi.org/10.1111/j.1478-3231.2006.01362.x

Takahashi, Y., & Fukusato, T. (2014). Histopathology of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World Journal of Gastroenterology*, *20*, 15539–15548. https://doi.org/10.3748/wjg.v20.i42.15539

Taylor, B., Rehm, J., & Gmel, G. (2005). Moderate alcohol consumption and the gastrointestinal tract. *Digestive Disease*, *23*, 170–176. https://doi.org/10.1159/000090163

Teli, M. R., Day, C. P., Burt, A. D., Bennett, M. K., & James, O. F. (1995). Determinants of progression to cirrhosis or fibrosis in pure alcoholic fatty liver. *Lancet, 346*, 987–990. https://doi.org/10.1016/s0140-6736(95)91685-7

Thakur, L., Kojicic, M., Thakur, S. J., Pieper, M. S., Kashyap, R., Trillo-Alvarez, C. A., Javier, F., Cartin-Ceba, R., & Gajic, O. (2009). Alcohol consumption and development of acute respiratory distress syndrome: a population-based study. *International Journal of Environmental Research and Public Health*, *6*, 2426–2435. https://doi.org/10.3390/ijerph6092426

Thalheimer, U., Triantos, C. K., Samonakis, D. N., Patch, D., & Burroughs, A. K. (2005). Infection, coagulation, and variceal bleeding in cirrhosis. *Gut*, *54*, 556–563. https://doi.org/10.1136/gut.2004.048181

Tonelo, D., Providência, R., & Gonçalves, L. (2013). Holiday heart syndrome revisited after 34 years. *Arquivos Brasileiros de Cardiologia*, *101*, 183–189. https://doi.org/10.5935/abc.20130153

Trias-Llimós, S., & Janssen, F. (2018). Alcohol and gender gaps in life expectancy in eight Central and Eastern European countries. *European Journal of Public Health*, *28*, 687–692. https://doi.org/10.1093/eurpub/cky057

Tsai, J., Ford, E. S., Li, C., & Zhao, G. (2012). Past and current alcohol consumption patterns and elevations in serum hepatic enzymes among US adults. *Addictive Behaviors, 37*, 78–84. https://doi.org/10.1016/j.addbeh.2011.09.002

Tsochatzis, E. A., Bosch, J., & Burroughs, A. K. (2014). Liver cirrhosis. *Lance, 383*, 1749–1761. https://doi.org/10.1016/S0140-6736(14)60121-5

Vezzoli, S., Bernini, M., & De Ferrari, F. (2015). Ethyl glucuronide in vitreous humor and blood postmortem specimens: analysis by liquid chromatography-electrospray tandem mass spectrometry and interpreting results of neo-formation of ethanol. *Annali Dell'Istituto Superiore Di Sanita*, *51*, 19–27. https://doi.org/10.4415/ANN_15_01_05

Waldthaler, A., Schütte, K., & Malfertheiner, P. (2010). Causes and mechanisms in acute pancreatitis. *Digestive Diseases, 28*, 364–372. https://doi.org/10.1159/000319416

Walsham, N. E., & Sherwood, R. A. (2014). Ethyl glucuronide and ethyl sulfate. *Advances in Clinical Chemistry*, *67*, 47–71. https://doi.org/10.1016/bs.acc.2014.09.006

Ware, J. S., Amor-Salamanca, A., Tayal, U., Govind, R., Serrano, I., Salazar-Mendiguchía, J., García-Pinilla, J. M., Pascual-Figal, D. A., Nuñez, J., Guzzo-Merello, G., Gonzalez-Vioque, E., Bardaji, A., Manito, N., López-Garrido, M. A., Padron-Barthe, L., Edwards, E., Whiffin, N., Walsh, R., Buchan, R. J., … Garcia-Pavia, P. (2018). Genetic etiology for alcohol-induced cardiac toxicity. *Journal of the American College of Cardiology*, *71*, 2293–2302. https://doi.org/10.1016/j.jacc.2018.03.462

Weintraub, R. G., Semsarian, C., & Macdonald, P. (2017). Dilated cardiomyopathy. *Lancet, 390*, 400–414. https://doi.org/10.1016/S0140-6736(16)31713-5

World Health Organization. (2019). *Global status report on alcohol and health 2018*. https://www.who.int/publications/i/item/9789241565639

World Health Organization. (2021). *Health for All database (HFA-DB)*. https://gateway.euro.who.int/en/datasets/european-health-for-all-database/

Wurst, F. M., Thon, N., Yegles, M., Schrück, A., Preuss, U. W., & Weinmann, W. (2015). Ethanol metabolites: their role in the assessment of alcohol intake. *Alcoholism, Clinical and Experimental Research*, *39*, 2060–2072. https://doi.org/10.1111/acer.12851

Yki-Järvinen, H. (2014). Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. *The Lancet. Diabetes and Endocrinology, 2*, 901–910. https://doi.org/10.1016/S2213-8587(14)70032-4

Zakhari, S. (2013). Bermuda Triangle for the liver: alcohol, obesity, and viral hepatitis. *Journal of Gastroenterology and Hepatology*, *28 Suppl 1*, 18–25. https://doi.org/10.1111/jgh.12207

Zakharov, S., Pelclova, D., Urban, P., Navratil, T., Diblik, P., Kuthan, P., Hubacek, J. A., Miovsky, M., Klempir, J., Vaneckova, M., Seidl, Z., Pilin, A., Fenclova, Z., Petrik, V., Kotikova, K., Nurieva, O., Ridzon, P., Rulisek, J., Komarc, M., & Hovda, K. E. (2014). Czech mass methanol outbreak 2012: Epidemiology, challenges and clinical features. *Clinical Toxicology, 52*, 1013–1024. https://doi.org/10.3109/15563650.2014.974106

Zhang, P., Bagby, G. J., Happel, K. I., Raasch, C. E., & Nelson, S. (2008). Alcohol abuse, immunosuppression, and pulmonary infection. *Current Drug Abuse Reviews*, *1*, 56–67. https://doi.org/10.2174/1874473710801010056

SUMMARY IN ESTONIAN

Alkoholist, psühhotroopsetest ja muudest ainetest põhjustatud surmad Eestis: kohtuarstlikel lahangutel põhinev tõendus

Eestis oli 2019. aastal keskmine oodatav eluiga meestel 74,4 ja naistel 82,8 aastat (Eesti Statistikaamet, 2021), mis on madalam ja mille sooline erinevus on suurem kui Euroopa Liidus keskmiselt. Oodatava eluea sooline lõhe on tingitud suurel määral tööealiste meeste liigsurmadest ning on sageli seotud alkoholi tarvitamisega.

Liigne alkoholi tarvitamine rahvastikus on üks peamistest enneaegsete surmade põhjustest (Jasilionis et al., 2020; Leon et al., 1997; Popova et al., 2007; Shield & Rehm, 2015). Liigsest alkoholi tarvitamisest põhjustatud enneaegne suremus on Eestis kõrge just meeste hulgas (Bogstrand et al., 2011; Rahu et al., 2019). Teine enneaegste surmade välditav põhjus Eestis on mürgistused psühhotroopsete ainetega.

Alkohol võib surma põhjustada või selle saabumist soodustada nii otseselt kui kaudselt (Bogstrand et al., 2011) ning on suuresti seotud kroonilise toimega siseorganitele (Britton & McKee, 2000; Lahti et al., 2011; Leon et al., 2010; Mäkelä, 1998; Persson et al., 2013). Muuhulgas sõltub alkoholi tarvitamisest põhjustatud surmade hulga ning jaotuse kajastamine surmapõhjuste kodeerimise tavast ning asjakohaste Rahvusvahelise Haiguste Klassifikatsiooni (RHK) koodide olemasolust.

Alkoholi pikaajalist tarvitamist ning sellest tingitud organkahjustusi tuvastavad kohtuarstid lahanguleiu, võimalusel lahkunud isiku tervishoiuteenuste dokumentide ja menetleja poolt antud eelandmete põhjal. Tervistkahjustava alkoholi tarvitamisega kaasnevad muutused laboratoorsetes näitajates nagu gammaglutamüüli transferaas (GGT), aspartaadi aminotransferaas (AST), alaniini aminotransferaas (ALT), erütrotsüüdi keskmine maht ja süsivesikdefitsiitne transferriin (CDT). Uuemad markerid uuritava subjekti poolt hiljutise alkoholi tarvitamise määramiseks on etüülglükuroniid (EtG) ja etüülsulfaat (EtS) ning pikemajalise alkoholi tarvitamise tuvastamiseks fosfatidüületanool (PEth). Alkoholi biomarkereid saab määrata ka surmajärgselt sellele eelnenud alkoholi tarvitamise kindlaks tegemiseks, kuid nende olulisust surmajärgses diagnostikas pole veel piisavalt uuritud.

Oluline on välja selgitada alkoholi roll inimese organismi kahjustamisel ning suurusjärk rahvastiku tervise mõjutamisel. Lahanguleidudel baseeruv uuring on üks viis selle teostamiseks. Taolised väärtuslikku informatsiooni andvad uuringud on keerukad ning seetõttu pole neid palju läbi viidud. Selleks, et tuua välja alkoholi roll surmade põhjustajana ning selle kajastamine rahvastiku surmade statistikas, on omakorda oluline hinnata ja vajadusel parendada surmapõhjuste kodeerimist.

Käesolev uuring käsitles tööealiste meeste alkoholist põhjustatud surmasid, kasutades selleks süvitsi minevat kohtuarstlike lahangute analüüsi, uuris põhihaiguse kodeerimisega seotud probleeme alkoholist põhjustatud hulgiorganite kahjustuse korral ning kirjeldas surmaga lõppenud alkoholi, psühhotroopsete ja muude ainete mürgistusi Eesti kogurahvastikus. Antud uuring on Eestis esimene, mis kasutab detailset kohtuarstlikku lahangut alkoholi rolli uurimiseks enneaegses suremuses.

Eesmärgid

Uurimistöö üldeesmärk oli anda tõenduspõhine ülevaade alkoholi, psühhotroopsete ja muude ainete tarvitamisega seotud surmadest ning kirjeldada alkoholi tarvitamisega seotud surmade registreerimist Eestis.

Töö alaeesmärgid olid järgmised:

1. Kirjeldada alkoholi ekspositsiooni, alkoholi biomarkereid ja peamisi surmapõhjuseid 25–54aastaste meeste hulgas aastatel 2008–2009 (I artikkel);
2. Välja selgitada alkoholi tarvitamisega seotud organkahjustusi ning analüüsida organkahjustuste seoseid alkoholi biomarkeritega 25–54aastaste meeste hulgas aastatel 2008–2009 (II artikkel);
3. Uurida peamiste surmapõhjuste kodeerimise probleeme alkoholi tarvitamisega seotud hulgiorgankahjustuste korral (II artikkel);
4. Analüüsida alkoholist, psühhotroopsetest ja muudest ainetest põhjustatud mürgitussurmasid kogurahvastikus aastatel 2000–2009 (III artikkel) ja 2010–2019.

Metoodika

Töö põhines kahel uuringul: Eesti tööealiste meeste alkoholi tarvitamisega seotud enneaegse suremuse uuring 2008–2009 (I uuring) ja Eesti kogurahvastiku mürgistussurmade uuring 2000–2009 ja 2010–2019 (II uuring).

I uuring

1. Uuritavad

Uuritavateks olid Eestis aastatel 2008–2009 kohtuarstlikule lahangule suunatud 25–54aastased mehed. Meeste kohtuarstlikke lahanguid teostati sel ajaperioodil kokku 2670, 1299 neist kuulusid vastavasse vanuserühma, kellest 605 kaasati uuringusse. Lõplikult oli sobivaid lahanguid 595, kuna kümnel juhul ei osutunud surnu vanus õigeks või jäi surnukeha tuvastamata (I artikkel). 545 lahangu puhul uuriti täpsemalt alkoholist põhjustatud organkahjustusi (II artikkel).

2. Kohtuarstliku lahangu läbiviimise protseduurid

Lahanguprotokoll ja histoloogiline uuring. Lahanguandmete kogumiseks kasutati spetsiaalselt välja töötatud lahanguprotokolli. Organite süstemaatilise uuringu tulemused, mis hõlmasid südame, neerude, kopsude, maksa, kõhunäärme, mao,

kaksteistsõrmiku ja peaaju nii makroskoopilist kirjeldust kui histoloogilist uuringut, märkis lahangu teinud kohtuarst vastavasse protokolli.

Bioloogilised proovid. Etanooli kontsentratsioon määrati veres, uriinis ning silma klaaskehavedelikus. Seerumis määrati maksaensüümide AST, ALT, GGT aktiivsus. Samuti määrati alkoholi metaboliitide kontsentratsioon: PEth veres ning EtG ja EtS uriinis.

Alkoholi toimest põhjustatud patoloogilised muutused. Meeste enneaegse suremuse uuringu tähelepanu keskmes olid alkoholi tarvitamisega seotud surmad, mistõttu määratleti kuus potentsiaalselt alkoholist põhjustatud patoloogiliste muutuste klassi vastavalt organitele (maks, kõhunääre, magu, söögitoru, kops ja süda), kus muutused esinesid. Organite alkoholikahjustusest tingitud patoloogilised muutused tehti kindlaks kombineeritult makroskoopilise vaatluse ja histoloogilise uuringu tulemuste alusel. Söögitoru veenilaiendid tuvastati ainult makroskoopiliselt. Iga patoloogiliste muutuste klassi esinemine või puudumine märgiti üles kahendmuutujana ilma vahepealsete variantideta.

3. Omaste intervjueerimine

Juhul, kui lahkunu oli elanud ühes viiest Eesti suuremast linnast (Tallinn, Tartu, Pärnu, Kohtla-Järve, Narva), küsitleti nende omakseid (n=169), et koguda detailseid andmeid eelkõige lahkunu alkoholi tarvitamise harjumuste kohta.

4. Põhihaiguse kodeerimine

Kohtuarstid märkisid lahanguprotokolli ning kodeerisid surma põhjuse ja kuni kolm kaasuvat haigust vastavalt RHK-10-le. Eesti surma põhjuste registrist saadi põhihaiguste RHK-koodid, mille võrdlemine kohtuarstide poolt omistatud koodidega võimaldas tuvastada kodeerimisel tekkivaid probleeme. Keskenduti järgmistele põhihaigustele: alkoholisõltuvus (F10.2), alkohoolne kardiomüopaatia (I42.6), maksa alkoholtõbi (K70), alkoholimürgistus (X45), kardiovaskulaarsed haigused (I00-I99, v.a. I42), teised kardiomüopaatiad (I42, v.a. I42.6), seedeelundite haigused (K00-K93, v.a. K70), hingamiselundite haigused (J00-J99) ning välispõhjused (V01-Y98, v.a. X45).

II uuring

Andmete kogumine*.* Uuring baseerus Eesti Kohtuekspertiisi Instituudi lahanguprotokollidest ja kohtukeemia laborist 2000–2009 aastate (III artikkel) ja 2010–2019 aastate kohta saadud andmetel (vanus, sugu, surma põhjus, toksikoloogia-uuringute tulemused). Uuringusse kaasati kõik lahangujuhud, kus põhihaiguseks oli mürgistus: aastatel 2000–2009 oli 4132 ja järgmisel kümnel aastal 2822 surmaga lõppenud mürgistust. RHK koodidest kasutati uuringus T jaotust vastavalt ainele (etanool, illegaalsed ained/ravimid, ravimid, teised alkoholid, süsinikmonooksiid, muud ja teadmata).

Tulemused

Alkoholi ekspositsioon, alkoholi biomarkerid ja peamised surmapõhjused 25–54aastaste meeste hulgas aastatel 2008–2009. (I artikkel)

Surmale eelnenud aasta jooksul olid tarvitanud alkoholi peaaegu iga päev või mitu korda nädalas üle poole tööealistest meestest ning umbes 10% neist olid mitmel korral tarvitanud surrogaatalkoholi. 55%-l juhtudest oli alkoholi sisaldus surnu veres 0,2 mg/g või enam, mis kinnitab nende liigset alkoholi tarvitamist.

Mitmed uudsed alkoholi biomarkerid osutusid sobivaks surmajärgses diagnostikas kasutamisel. Kõrge spetsiifilisusega olid surmajärgselt määratuna PEth veres ning EtG ja EtS uriinis. Biomarkerite väärtused olid nende isikute proovides negatiivsed või madalad, kes ei tarvitanud surmale eelnenud aasta jooksul alkoholi üldse või peaaegu mitte kunagi.

Eesti tööealiste meeste surma põhjustest moodustasid ligikaudu kaks kolmandikku välispõhjused ning ühe kolmandiku haigused. Välispõhjustest omakorda olid ülekaalus enesetapud (25,6%) ja mürgistused (24,8%). Haigustest olid surma põhjuseks pooltel juhtudel südame-veresoonkonnahaigused (eeskätt südame isheemiatõbi) ning sageduselt järgmiseks seedeelundite haigused (põhiliselt maksa alkoholtõbi).

Alkoholi tarvitamisega seotud organkahjustused ning selle seosed alkoholi biomarkeritega 25–54aastaste meeste hulgas aastatel 2008–2009. (II artikkel)

Alkoholist põhjustatud siseelundite patoloogiliste muutuste leid oli sage, mis kinnitab kohtuarstlikule lahangule suunatud 25–54aastaste meeste tervist ohustavat alkoholi tarvitamist. Ainult veerandil juhtudest ei tuvastatud lahangul alkohoolsele kahjustusele viitavaid organkahjustusi. Kahe või enama organi kahjustus esines 32%-l juhtudest ning kahjustatud organite arv suurenes vanuse kasvades, olles kõrgem vanuserühmas 45–54 aastat. Organite lõikes oli kõige sagedamini kahjustatud maks (orienteeruvalt 60% juhtudest). Võimalikud alkoholiga seotud patoloogilised muutused, nt pneumoonia ja aspiratsioon, esinesid 18,6%, mao ja söögitoru kahjustused 18,9% juhtudest ning äge või krooniline pankreatiit sedastati 14% juhtudest.

Biomarkeritest olid PEth ja GGT tõusnud väärtused seotud suurema arvu kahjustunud organitega. Samas EtG ja EtS väärtused korreleerusid mistahes organkahjustuse olemasolu või nende täieliku puudumisega.

Peamiste surmapõhjuste kodeerimise probleemid alkoholi tarvitamisega seotud hulgiorgankahjustuse korral (II artikkel)

Arstil on raske valida ühe organi alkoholist põhjustatud patoloogiat põhihaiguseks juhul, kui tegelikult esineb mitme organi kahjustus. Sellisel juhul peavad Eesti kohtuarstid õigeks diagnoosi “Alkoholi krooniline toksiline toime siseorganitesse”. Uuring näitas, et see diagnoos kodeeriti Statistikaametis koodiga F10.2 (alkoholsõltuvus), mis on pigem kliiniline diagnoos ning pole sobiv

kasutamiseks lahangujuhtudel. Kuna kõige sagedamini on hulgiorganpatoloogiate korral kahjustunud maks, siis kasutavad kohtuarstid enim diagnoosi K70 ehk maksa alkoholtõbi, kuigi see pole alati täiesti korrektne.

Alkoholist, psühhotroopsetest ja muudest ainetest põhjustatud mürgitussurmad kogurahvastikus aastatel 2000–2009 (III artikkel) ja 2010–2019

Kõigist surmadest Eestis moodustasid uuringuperioodil 2000–2009 mürgistused 2,4%. Kõige rohkem esines etanoolist põhjustatud mürgistussurmi, millele järgnesid mürgistused süsinikmonooksiidi ja narkootiliste ainetega. Sellel perioodil oli märgatav alkoholimürgistuste arvu langus ning viimastel aastatel ka vingumürgistuste vähenemine. Narkootiliste ainete mürgistustest moodustasid põhiosa (65,3%) mürgistused fentanüülidega. Orienteeruvalt 6% mürgistustest olid põhjustatud metadooni toksilisest toimest, samas ei sedastatud ühtegi surma, mis oleks põhjustatud buprenorfiinist.

Aastatel 2000–2009 moodustasid mono-intoksikatsioonid, peamiselt alkoholimürgistused üle poole kõigist letaalsetest mürgistustest. Vingugaasist ning narkootilistest ainetest tingitud surmad olid sageli kombineeritud mürgistused mitme ainega, neist narkosurmade puhul leiti kaasuvana sageli bensodiasepiine ning amfetamiini. 61%-l narkosurmadest oli alkoholi kontsentratsioon veres alla 0,2 mg/g.

Võrreldes eelneva kümnendiga oli aastatel 2010–2019 mürgistussurmade arv väiksem, samas oli langenud ka Eesti Kohtuekspertiisi Instituudis teostatud lahangute üldarv. Mürgistuste osakaalus kõigist kohtuarstlikest lahangutest esines vähene tõus. Kui aastatel 2000–2009 moodustasid mürgistused 14,3% kõigist lahangutest, siis järgmisel kümnel aastal moodustas see 17,6%. Märkimisväärne langus esines vingumürgistustes – aastatel 2010–2019 vähenes nende arv võrreldes eelmise kümnendiga ligikaudu kahe kolmandiku võrra.

Järeldused

Uuring andis tõenduspõhise ja detailse ülevaate, et Eestis kohtuarstlikule lahangule suunatud tööealistel meestel esinevad sageli võimalikud alkoholist põhjustatud haiguslikud muutused siseorganites, mis viitab selles vanuserühmas alkoholi harjumuspärasele ja tervistkahjustavale tarvitamisele. Uuringu tulemustest nähtus, et osad alkoholi biomarkerid (PEth, EtG ja EtS) on kasutatavad surmajärgses diagnostikas ning neid võiks tavapraktikas ja lahanguid hõlmavates uuringutes enam kasutada, eriti kui puuduvad lähedastelt saadud andmed lahkunu alkoholi tarvitamise mustri kohta.

Lahanguleiuna hulgiorganite alkoholkahjustuse esinemise korral oli raske diagnoosida põhihaigust RHK-s puuduvate võimaluste tõttu.

Mürgistussurmade uuringu põhjal olid aastatel 2000–2009 esikohal alkoholimürgistused kuid aastatel 2010–2019 mürgistused psühhotroopsete ja narkootiliste ainetega.

Praktilised soovitused

Uuringu tulemused on vajalikud alkoholistrateegia ja -poliitika kujundajatele ning Eesti Kohtuekspertiisi Instituudile kui kohtuarstlikke lahanguid teostavale asutusele.

Uuringu tulemuste põhjal saab teha järgmised ettepanekud:

1. Kasutada surma järgselt laialdasemalt alkoholi biomarkerite (Etg, Ets ja PEth) määramist kroonilise alkoholi tarvitamise ja/või vahetult enne surma toimunud alkoholi tarvitamise tõestamiseks.
2. Arendada ja juurutada neuropatoloogiat alkoholist põhjustatud organkahjustuste detailsemaks uurimiseks kohtuarstlikus praktikas
3. Märkida alkoholist põhjustatud hulgiorgankahjustuse korral surma põhjuse teatisele põhihaigusena juhtiva organi kahjustus.
4. Kasutada sagedamini beetahüdroksübutüraati kui väljendunud ketoatsidoosi näitajat otsese surmapõhjuse määramiseks juhul kui on tegemist alkoholist põhjustatud hulgiorgankahjustusega.
5. Parandada mürgistussurmade andmebaasi kvaliteeti Eestis regulaarse andmekontrolli ja lahanguprotokollide sisu võrdlusega.

APPENDIX I: Autopsy protocol

Study subject number	
Forensic autopsy protocol number	
Forensic department number	

Date and time

1	Date of death		
2	Time of death	:	
3	Date of autopsy		
4	Time of autopsy	:	
5	Date blood sample taken		
6	Time blood sample taken	:	

Instructions for completing the protocol

Please circle the appropriate number in front of the text answer.

Please record numeric data on the line provided.

Please write free text answers in Histology section.

Autopsy findings

Heart

7	Weight (g)	.
8	Length (cm)	....
9	Breadth (cm)	....
10	Depth (cm)	....
11	Thickness of left ventricular wall (cm)	....
12	Thickness of right ventricular wall (cm)	....
13	Thickness of intraventricular septum (cm)	....

Evidence of acute infarction			
14	Anterior		
		0	no
		1	yes
15	Posterior		
		0	no
		1	yes
16	Intraventricular septum		
		0	no
		1	yes
17	Apical		
		0	no
		1	yes
Evidence of old infarction			
18	Anterior		
		0	no
		1	yes
19	Posterior		
		0	no
		1	yes
20	Intraventricular septum		
		0	no
		1	yes
21	Apical		
		0	no
		1	yes
Other gross cardiac anomaly			
22	Dilated (cause unspecified) cardiomyopathy		
		0	no
		1	yes
23	Alcoholic cardiomyopathy		
		0	no
		1	yes

24	Hypertrophic cardiomyopathy		
		0	no
		1	yes
25	Chronic ischaemic heart disease		
		0	no
		1	yes

Atheromatous occlusion of coronary arteries

26	Left circumflex		
		0	none
		1	< 50%
		2	51-75%
		3	> 75%
27	Left anterior descending		
		0	none
		1	< 50%
		2	51-75%
		3	> 75%
28	Right coronary		
		0	none
		1	< 50%
		2	51-75%
		3	> 75%

Appearance of arteries of coronary arteries

29	Left circumflex		
		0	clean
		1	fatty streak +/- fibrosis
		2	complicated
		3	calcified
30	Left anterior descending		
		0	clean
		1	fatty streak +/- fibrosis
		2	complicated
		3	calcified

31	Right coronary		
		0	clean
		1	fatty streak +/- fibrosis
		2	complicated
		3	calcified

Thrombus of coronary arteries

32	Left circumflex		
		0	no
		1	yes
33	Left anterior descending		
		0	no
		1	yes
34	Right coronary		
		0	no
		1	yes

Other cardiovascular system

35	Anomaly of cardiac valves		
		0	no
		1	yes
36	End organ damage from hypertension		
		0	no
		1	yes
37	Renal damage from hypertension		
		0	no
		1	yes
38	Aorta		
		0	clean
		1	fatty streak +/- fibrosis
		2	complicated
		3	calcified

Kidneys

39	Renal disease		
		0	no
		1	yes

Lungs

40	Pneumonia		
		0	no
		1	yes
41	Aspiration of gastric contents		
		0	no
		1	yes
42	Active tuberculosis		
		0	no
		1	yes
43	Healed tuberculosis with scarring		
		0	no
		1	yes

Liver

44	Weight (g)	....
45	Right lobe height (cm)	....
46	Right lobe depth (cm)	....
47	Left lobe height (cm)	....
48	Left lobe depth (cm)	....
49	Length (cm)	....

Liver pathology

50	Liver pathology		
		0	none
		1	fatty liver (focal)
		2	fatty liver (generalised)
		3	fibrosis (incomplete)
		4	fibrosis (complete)
		5	cirrhosis (incomplete)
		6	cirrhosis (complete)
		7	morbis hepatis

Pancreatitis

51	Pancreas pathology		
		0	normal
		1	acute pancreatitis
		2	chronic pancreatitis

Oesophagus, stomach and duodenum

52	Gastritis		
		0	no
		1	yes
53	Gastric ulcer		
		0	no
		1	yes
54	Duodenitis		
		0	no
		1	yes
55	Duodenal ulcer		
		0	no
		1	yes
56	Oesophageal varicosities		
		0	no
		1	yes
57	Oesophagitis		
		0	no
		1	yes

Brain

58	Weight (g)	....

Brain pathology

59	Haemorrhage		
		0	no
		1	yes
60	Infarction		
		0	no
		1	yes

61	New contusions		
		0	no
		1	yes
62	Old brain trauma		
		0	no
		1	yes
63	Cortical atrophy		
		0	no
		1	yes
64	Cerebellar atrophy		
		0	no
		1	yes

Laboratory investigations

Blood, vitreous humour and urine alcohol level

65	Blood (mg/ml)	....
66	Urine	....
67	Vitreous humour (mg/ml)	....

Surrogates

		Concentration	
		Blood	**Urine**
68	Methanol	....	....
69	Iso-propanol	....	....
70	Iso-butanol	....	....
71	Iso-amyl alcohol	....	....
72	Ethylene Glycol	....	....

Drugs

				Detected	**Blood concentration**
73	Morphine				
		0	no		
		1	yes		....
		9	not tested		

74	Fentanyl		
		0 no	
		1 yes	....
		9 not tested	
75	Marijuana		
		0 no	
		1 yes	....
		9 not tested	
76	Amphetamine		
		0 no	
		1 yes	....
		9 not tested	
77	Cocaine		
		0 no	
		1 yes	....
		9 not tested	
78	Buprenorphine		
		0 no	
		1 yes	
		9 not tested	
79	Methadone		
		0 no	
		1 yes	....
		9 not tested	
80	Other, specify		
		0 no	
		1 yes	....
		9 not tested	

Enzymes etc

	Substance	**Concentration**
81	γ GT	....
82	Alanine Transferase	....
83	Aspartine Transferase	....

Histology

Heart

84	Right ventriculum	.....
85	Left ventriculum	.....
86	Septum intraventricularis	.....
87	Left atrium	.....
88	Right atrium	.....

Coronary artery

89	In case of thrombus	.....

Liver

90	Right	.....
91	Left	.....

Pancreas

92	Head	.....
93	Tail	.....

Brain

94	Cortex	.....
95	Cerebellum (superior vermis)	.....
96	Basal ganglia	.....
97	Mamiliary bodies	.....

Kidneys

98	Right	.....
99	Left	.....

Lung

100	Parenchyma	.....
101	Any lesion	.....

Stomach & duodenum

102	Lesion	.....

Cause of death

To be completed once death certificate issued

103	Cause of death (ICD10)	[ ][ ][ ] . [ ]
104	Immediate cause	.....
105	Intermediate cause	.....
106	Main cause	.....
107	External causes	.....
108	Other important diseases (1)	.....
109	Other important diseases (2)	.....
110	Other important diseases (3)	.....
111	Manner of death	

1 disease

2 suicide

3 accident

4 homicide

5 unknown

6 other

APPENDIX II: Participant proxy questionnaire

Participant proxy questionnaire
Cover Sheet: to be completed by the interviewer

Subject number | | | | | | |

Date of interview MM | | | YYYY | | | | |

Date of previous interview MM | | | YYYY | | | | |

Interviewer first name .....

Interviewer last name .....

Interviewer code | | |

Time started | | | : | | |

Time ended | | | : | | |

Having read the information sheet, are you willing to be interviewed and for the information collected to be used for the purposes of this scientific study?

Has respondent read the study information sheets? Yes | |

Has respondent given verbal consent? Yes | |

I am now going to ask you a series of questions regarding drinking of alcohol by *the subject*. These questions are about the past year, unless otherwise specified

Surrogates are mentioned in the following questions. These are substances not intended for drinking, including eau de colognes and medicinal tinctures as well as other things. They may be found in shops, chemists and kiosks.

For each type of drink listed in the left hand column, please indicate how often each is usually drunk

	every day or more often	nearly every day	3-4 times a week	once/ twice a week	1-3 times a month	a few times per year	never/ almost never	difficult to answer	refuse to answer
L0e alcohol (beer, wine, spirits or anything else containing alcohol)	1	2	3	4	5	6	7	97	98
L5 beer	1	2	3	4	5	6	7	97	98
L6 wine	1	2	3	4	5	6	7	97	98
L7 spirits	1	2	3	4	5	6	7	97	98
L8 surrogates	1	2	3	4	5	6	7	97	98
L0f homemade samogon	1	2	3	4	5	6	7	97	98
L0g homemade wine	1	2	3	4	5	6	7	97	98

For each type of drink listed in the left column, please indicate on which day of the wee each is usually drunk

	only at the weekend	only on holidays/ celebrations	on no par-ticular day	never/almo st never	difficult to answer	refuse to answer
L0e alcohol (beer, wine, spirits or anything else containing alcohol)	1	2	3	4	97	98
L5 beer	1	2	3	4	97	98
L6 wine	1	2	3	4	97	98
L7 spirits	1	2	3	4	97	98
L8 surrogates	1	2	3	4	97	98
L0f homemade samogon	1	2	3	4	97	98
L0g homemade wine, braga	1	2	3	4	97	98
L0h alcoholic cocktails (premixed bottles)	1	2	3	4	97	98

Interviewer! Skip to L9

L0i. Has he ever drunk alcohol in his life other than on a few occasions?

Please circle the single most appropriate answer.

1 Yes → ***go to*** *L35a*
2 No → ***go to*** *L46*
97 difficult to answer → ***go to*** *L46*
98 refuse to answer → ***go to*** *L46*

L9. How much beer does he usually drink on one occasion? ('occasion' means a single continuous period of drinking)

Please circle the single most appropriate answer.

1 never drinks beer
2 1 bottle (0.5l) or less
3 2-4 bottles (0.5l)
4 5-6 bottles (0.5l)
5 more than 6 bottles(0.5l)
97 difficult to answer
98 refuse to answer

L10. How much wine does he usually drink on one occasion?

Please circle the single most appropriate answer.

1 never drinks wine
2 up to 200g
3 between 200 - 400g
4 between 400 - 600g
5 between 600 - 1000g
6 more than 1 litre
97 difficult to answer
98 refuse to answer

L11. What quantity of spirits, such as vodka or other strong drinks, does he usually drink on one occasion?

Please circle the single most appropriate answer.

1 never drinks spirits
2 Up to 50g
3 between 50 – 100g
4 between 100 - 200g
5 between 200 - 300g
6 between 300 - 400g
7 between 400 - 500g
8 more than 500g
97 difficult to answer
98 refuse to answer

L12. What is the maximum quantity of beer ever drunk on one occasion?

Please circle the single most appropriate answer.

1 never drinks beer
2 1 bottle (0.5l) or less
3 2-4 bottles (0.5l)
4 5-6 bottles (0.5l)
5 more than 6 bottles (0.5l)

97 difficult to answer
98 refuse to answer

L13. What is the maximum quantity of wine ever drunk on one occasion?

Please circle the single most appropriate answer.

1 never drinks wine
2 up to 200g
3 between 200 - 400g
4 between 400 - 600g
5 between 600 - 1000g
6 more than 1 litre
97 difficult to answer
98 refuse to answer

L14. What is the maximum quantity of spirits ever drunk on one occasion?

Please circle the single most appropriate answer.

1 never drinks spirits
2 up to 50g
3 between 50 – 100g
4 between 100 - 200g
5 between 200 - 300g
6 between 300 - 400g
7 between 400 - 500g
8 more than 500g
97 difficult to answer
98 refuse to answer

L15. Does he ever drink spirits together with either beer or wine at the same sitting?

Please circle the single most appropriate answer.

1 yes, often
2 yes, sometimes
3 no, never
97 difficult to answer
98 refuse to answer

L16. Does he ever drink large quantities of spirits without also eating some food at the same sitting?

Please circle the single most appropriate answer.

1 always
2 sometimes
3 rarely/never
97 difficult to answer
98 refuse to answer

L17. How often does he become excessively drunk?

Please circle the single most appropriate answer.

1 every day
2 several times a week
3 once a week
4 several times a month
5 once a month
6 less than once a month
7 never or almost never
97 difficult to answer
98 refuse to answer

L18. Does he ever drink alcohol before noon?

Please circle the single most appropriate answer.

1 no
2 yes, occasionally
3 yes, frequently
97 difficult to answer
98 refuse to answer

L19. How often does he have a hangover?

Please circle the single most appropriate answer.

1 every day
2 several times a week
3 about once a week
4 several times a month
5 about once a month
6 less than once a month
7 never or almost never
97 difficult to answer
98 refuse to answer

L20. This question is deliberately omitted

L20a During the last month, were they any days when he missed work because he felt unwell due to alcohol?

Please circle the single most appropriate answer.

1 Yes
2 No → ***go to*** *L21*
3 Did not work → ***go to*** *L21*
97 difficult to answer → ***go to*** *L21*
98 refuse to answer → ***go to*** *L21*

L20b If yes, approximately how many days?

☐☐ days

97 difficult to answer
98 refuse to answer

L21. How often does he fail to fulfil his family or personal obligations due to drinking alcohol?

1 every day
2 several times a week
3 about once a week
4 several times a month
5 about once a month
6 less than once a month
7 Never
97 difficult to answer
98 refuse to answer

L22. Does he ever go to sleep at night with his clothes on because of being drunk?

Please circle the single most appropriate answer.

1 every day
2 several times a week
3 about once a week
4 several times a month
5 about once a month
6 less than once a month
7 never or almost never
97 difficult to answer
98 refuse to answer

L23. Does he ever drink alone?

Please circle the single most appropriate answer.

1 yes, often
2 yes, sometimes
3 no, never
97 difficult to answer
98 refuse to answer

L24. Does he usually drink alcohol at home or in other places?

Please circle the single most appropriate answer.

1 usually at home
2 sometimes at home, sometimes elsewhere
3 usually elsewhere
97 difficult to answer
98 refuse to answer

L24a With whom does he usually drink?

Multiple responses are permitted.

1 with the members of your household
2 with other relatives
3 with friends who work with you
4 with neighbours
5 with friends from childhood (youth)
6 with friends you know through your hobbies
7 a variety of people
8 usually drinks alone
9 other
97 difficult to answer
98 refuse to answer

I would now like to ask you about episodes of 'zapoi' in *the subject's* life. By 'zapoi', I mean a period of continuous drunkeness of several days or more during which the person does not work and is withdrawn from normal life.

L25. Has he had one or more episodes of zapoi in the past <u>year</u>?

Please circle the single most appropriate answer.

1 yes, often had episodes of zapoi
2 yes, sometimes had episodes of zapoi
3 no, never)
97 difficult to answer) go to L32
98 refuse to answer)

L26. Has he had one or more episodes of zapoi in the past <u>month</u>?

Please circle the single most appropriate answer.

1 yes
2 no)
97 difficult to answer) go to L27b
98 refuse to answer)

L27. Has he had one or more episodes of zapoi in the past <u>week</u>?

Please circle the single most appropriate answer.

1 yes
2 no
97 difficult to answer
98 refuse to answer

L27b How long does a typical episode last?

Please circle the single most appropriate answer.

1 2 days
2 3 days
3 4 or more days
97 difficult to answer
98 refuse to answer

L27c How many episodes has he had in the past year?

Please circle the single most appropriate answer.

1 1
2 2-4
3 5-9
4 10 or more
97 difficult to answer
98 refuse to answer

L28. This question is deliberately omitted

L29. This question is deliberately omitted

L30. This question is deliberately omitted

L31. During his most recent episode of zapoi, did he drink surrogates (any alcoholic substances not intended for drinking) ?

Please circle the single most appropriate answer.

1 yes
2 no
97 difficult to answer
98 refuse to answer

L32. Has he been arrested because he was drunk during the past year?

Please circle the single most appropriate answer.

1 yes
2 no
97 difficult to answer
98 refuse to answer

L33. Is he currently drinking more than, less than, or about the same as he was one year ago?

Please circle the single most appropriate answer.

1 more than a year ago ***go to*** L35a
2 about the same as a year ago ***go to*** L35a
3 less than a year ago
97 difficult to answer ***go to*** L35a
98 refuse to answer ***go to*** L35a

L33b Is this because of…

Please circle the single most appropriate answer.

1 He was afraid of losing his job
2 Advised by doctor to stop
3 After treatment for alcohol problems
4 Felt too ill to drink
5 Pressure from or influence of his family or friends
6 Financial reasons
7 He decided he didn't want to drink alcohol any more for other health-/illness-related reasons (please specify)…… ……
8 He decided he didn't want to drink alcohol any more for other non health-/illness-related reasons (please specify)…… ……
97 difficult to answer
98 refuse to answer

L34. This question is deliberately omitted

L35a Has he drunk <u>any</u> alcohol in the past month?

Please circle the single most appropriate answer.

1 Yes ***go to*** L35
2 No
97 difficult to answer ***go to*** L35
98 refuse to answer ***go to*** L35

L35b When did he stop drinking alcohol?

Please circle the single most appropriate answer.

1 up to 6 months ago
2 more than 6, up to 12 months ago
3 more than 1, up to 5 years ago
4 more than 5 years ago
97 difficult to answer
98 refuse to answer

L35c Why did he stop drinking alcohol?

Please circle the single most appropriate answer.

1 He was afraid of losing his job
2 Advised by doctor to stop
3 After treatment for alcohol problems
4 Felt too ill to drink
5 Pressure from or influence of his family or friends
6 Financial reasons
7 He decided he didn't want to drink alcohol any more for other health-/illness-related reasons (please specify)…… ……
8 He decided he didn't want to drink alcohol any more for other non health-/illness-related reasons (please specify)…… ……
97 difficult to answer
98 refuse to answer

L35. Was there ever any period in his life when he drank heavily other than during the past 12 months?

Please circle the single most appropriate answer.

1 yes
2 No
97 difficult to answer
98 refuse to answer

L36. Has he ever had help or advice from a doctor, narcologist, social worker or some other professional for an alcohol problem?

Please circle the single most appropriate answer.

1 yes
2 no
97 difficult to answer ***go to*** *L38*
98 refuse to answer

L37. Did he get such help or advice in the past 12 months?

Please circle the single most appropriate answer.

1 yes
2 no
97 difficult to answer
98 refuse to answer

L37b Has he ever attended the Narcology Dispensary?

Please circle the single most appropriate answer.

1 Yes
2 No
97 difficult to answer
98 refuse to answer

L38. Has he ever been taken to a sobering-up centre?

Please circle the single most appropriate answer.

1 yes
2 no
97 difficult to answer ***go to*** *L41*
98 refuse to answer

L39. Was this during the past 12 months?

Please circle the single most appropriate answer.

1 yes
2 no
97 difficult to answer
98 refuse to answer

L40. This question is deliberately omitted

L41 How long, in minutes, does it take to get to the nearest place where one can buy beverages?

Please circle the single most appropriate answer.

1 <5 minutes
2 5-10 minutes
3 10-30 minutes
4 >30 minutes
97 Difficult to answer
98 refuse to answer

L44 Has he ever been admitted to hospital/clinic because of alcohol poisoning?

Please circle the single most appropriate answer.

1 Yes
2 No ***go to*** *L46*
97 Difficult to answer ***go to*** *L46*
98 refuse to answer ***go to*** *L46*

L45 What had he drunk?

…….

97 Difficult to answer
98 refuse to answer

L46 Did his father go on zapoi when he was growing up?

Please circle the single most appropriate answer.

1 Yes
2 No
97 Difficult to answer
98 refuse to answer

L47 Did his father drink surrogates when he was growing up?

Please circle the single most appropriate answer.

1 Yes
2 No
97 Difficult to answer
98 refuse to answer

L48 Does anyone in the participant's household apart from him go on zapoi?

Please circle the single most appropriate answer.

1 Yes
2 No
97 Difficult to answer
98 refuse to answer

L49 Does anyone in the participant's household apart from him drink surrogates?

Please circle the single most appropriate answer.

1 Yes
2 No
97 Difficult to answer
98 refuse to answer

Interviewer: ask the following questions to proxies of men who drink surrogates a few times per year or more often

S1 What is the main reason that he drinks surrogates?

Please circle the single most appropriate answer.

1 Taste
2 Psychological /physical effect
3 Ease of purchase
4 Price
5 Other reasons. Please specify ……..
97 difficult to answer
98 refuse to answer

S2 When did he start consuming surrogates?

Please circle the single most appropriate answer.

1 within the past month
2 within past 6 months
3 within the last year
4 more than a year ago
97 difficult to answer
98 refuse to answer

S3 What surrogates does he drink?

Interviewer! Please show respondent card S3.

Select all possible answers

1 Yason
2 Troyar
3 Composition
4 Troynoy or any other cologne or perfume (write what exactly he consumes) _____
5 Infusion of juniper
6 Infusion of hawthorn
7 Pepper tincture
8 Other types of spirituous infusions (what exactly) _____
9 Spirits (technical, medical or other)
10 Windows cleaning liquid, other cleaners
Other types of liquids containing spirits (which exactly?) _____
97 difficult to answer
98 refuse to answer

S4 Does he ever drink surrogates at home?

Please circle the single most appropriate answer.

1 Yes
2 No
97 difficult to answer
98 refuse to answer

S5 Where does he usually buy surrogates?

Please circle the single most appropriate answer.

1 Kiosk
2 Pharmacy
3 Market
4 Other shop
97 difficult to answer
98 refuse to answer

S6 How long time does it take him to get to the nearest place that he buys surrogates?

Please circle the single most appropriate answer.

1 <5 minutes
2 5-10 minutes
3 10-30 minutes
4 >30 minutes
97 difficult to answer
98 refuse to answer

S7 When he drinks surrogates, how many bottles does he usually drink per day?

_____bottles
97 difficult to answer
98 refuse to answer

S8 When he drinks surrogates, how much in mls does he usually drink a day? (not diluted)

_____mls

97 difficult to answer

98 refuse to answer

S9 How do you know that the participant consumes surrogates?

(mark all possible answers)

1 he drank openly (in front of you, told you about it)

2 you bought surrogates for him or gave him money to buy surrogates

3 you found empty bottles, flacks

4 you could smell that he had

5 someone told you

6 other (please specify)_____

97 difficult to answer

98 refuse to answer

S10 What best desribes his drinking behaviour before he started using surrogates?

Please circle the single most appropriate answer.

1 drank beverages, but not very much

2 drank beverages a lot but had no zapoi

3 drank beverages a lot and went on zapoi

97 difficult to answer

98 refuse to answer

ACKNOWLEDGEMENTS

The work was carried out at the Department of Pathological Anatomy and Forensic Medicine and the Institute of Family Medicine and Public Health, University of Tartu. The study was funded by the Estonian Science Foundation (grant number 8847) and the Wellcome Trust (grant number 078557).

I would like to express my deepest thanks to my supervisors.

Professor Marika Väli for the mentorship, belief in me when I was lost, and encouragement. Associate Professor Kersti Pärna for her patience, punctuality and devotion while supervising the work and delicately pushing me towards the goal. Associate Professor Katrin Lang for sharing her knowledge about scientific work and enriching my vocabulary.

This work would not have been possible without Professor David A Leon and Professor Martin McKee from the London School of Hygiene and Tropical Medicine who initiated the study and generated brilliant ideas. My special thanks to Professor Leon for reviewing this work.

I am very grateful to Professor Andres Arend and Associate Professor Kadri Suija for reviewing this thesis and giving constructive feedback.

I would also like to acknowledge the help and collaboration of Gleb Denissov, Head of Estonian Causes of Death Registry.

I am very thankful to statistician Inge Ringmets for collaboration and performing data analysis.

I thank all the doctors of the Estonian Forensic Science Institute who participated in the study of premature death. And my special thanks belong to my dear colleague Mailis Tõnisson as the co-author of papers and the expert of forensic medical toxicology.

Finally, I am most thankful to my daughter Kea, and also to Tanel with his family for their never-ending support in different forms, and love.

PUBLICATIONS

CURRICULUM VITAE

Name: Jana Tuusov
E-mail: jana.tuusov@ekei.ee

Education

2007–…	Doctoral studies, Faculty of Medicine, University of Tartu
1998–2002	Residency in pathology, Faculty of Medicine, University of Tartu
1996–1997	Internship, Faculty of Medicine, University of Tartu
1989–1996	Faculty of Medicine, University of Tartu
1978–1989	Tartu M. Härma Secondary School No 2.

Professional employment

2017–…	Head of the Southern Estonian Forensic Medical Department, Estonian Forensic Science Institute
2008–2017	Forensic medical expert, Estonian Forensic Science Institute
2002–2008	Forensic medical expert, Estonian Bureau of Forensic Medicine

Scientific work

Research field:

Deaths caused by alcohol, narcotic and psychotropic substances.

Participation in research projects:

- Estimation of time since death (*post mortem* interval). Significance of biochemical investigation (2013–2016, PUT 98)
- Genetic correlates of gene and protein expression endophenotypes in the brain areas of schizophrenic patients (2013–2016, PUT 129)

Membership: Estonian Association of Forensic Doctors
Tartu Medical Association

Publications

Ringmets, I., Tuusov, J., Lang, K., Väli, M., Pärna, K., Tõnisson, M., Helander, A., McKee, M., & Leon, D.A. (2012). Alcohol and premature death in Estonian men: a study of forensic autopsies using novel biomarkers and proxy informants. *BMC Public Health*, *12,* 146.

Tuusov, J., Lang, K., Väli, M., Pärna, K., Tõnisson, M., Ringmets, I., McKee, M., Helander, A., & Leon, D.A. (2014). Prevalence of alcohol-related pathologies at autopsy: Estonian Forensic Study of Alcohol and Premature Death. *Addiction, 109*, 2018–26.

Tuusov, J., Vals, K., Tõnisson, M., Riikoja, A., Denissov, G., & Väli, M. (2012). Fatal poisoning in Estonia 2000-2009. Trends in illegal drug-related deaths. *Journal of Forensic and Legal Medicine, 20,* 51–6.

Törő, K., Väli, M., Lepik, D., Tuusov, J., Dunay, G., Marcsa, B., Pauliukevicius, A., Raudys, R., & Caplinskiene, M. (2013). Characteristics of cardio-

vascular deaths in forensic medical cases in Budapest, Vilnius and Tallinn. *Journal of Forensic and Legal Medicine, 20,* 968–71.
Töro, K., Szilvia, F., György, D., Pauliukevicius, A., Caplinskiene, M., Raudys, R., Lepik, D., Tuusov, J., Väli, M. (2011), Fatal traffic injuries among children and adolescents in three cities (capital Budapest, Vilnius, and Tallinn). *Journal of Forensic Sciences, 56,* 617–20.
Väli, M., Tuusov, J., Lang, K. & Pärna, K. (2011). Child abuse and the external cause of death in Estonia. In: Duarte Nuno Vieira (Ed.). Forensic Medicine from old problems to new challenges. Croatia: InTech – Open Access Publisher, pp. 178−88.

Professional development

During past years attended at several international conferences with poster (BMLA 2010, 2014 ISALM, 2016 IALM) and oral presentations (BMLA 2017). Since 2017 the head of the Southern Estonian Forensic Medical Department. In 2019 acquired professional standard – forensic medical doctor, EstQF level 7.

ELULOOKIRJELDUS

Nimi: Jana Tuusov
E-post: jana.tuusov@ekei.ee

Haridus

2007–…	Tartu Ülikooli arstiteaduskond, doktoriõpe
1998–2002	Tartu Ülikooli arstiteaduskond, patoloogia residentuur
1996–1997	Tartu Ülikooli arstiteaduskond, internatuur
1989–1996	Tartu Ülikooli arstiteaduskond, ravi eriala
1978–1989	M. Härma nim. Tartu 2. Keskkool

Teenistuskäik

2017–…	Eesti Kohtuekspertiisi Instituut, Lõuna-Eesti kohtuarstliku ekspertiisiosakonna juhataja
2008–2017	Eesti Kohtuekspertiisi Instituut, kohtuarst-ekspert
2002–2008	Eesti Kohtuarstlik Ekspertiisibüroo, kohtuarst-ekspert

Teadustöö

Uurimisvaldkond:
Alkoholi ning narkootiliste ja psühhotroopsete ainete tarvitamisest tingitud surmad
Osalenud teadusprojektides:

- Surmast möödunud ajavahemiku (postmortaalse intervalli) hindamine. Biokeemiliste uuringute tähtsus (2013–2016, PUT 98)
- Geneetiliste korrelaatide leidmine geeni ja valgu ekspressioonitasemega seotud endofenotüüpidele skisofreeniapatsientide ajuosades (2013−2016, PUT 129)

Liikmelisus: Eesti Kohtuarstide Selts
Tartu Arstide Liit

Publikatsioonid

Ringmets, I., Tuusov, J., Lang, K., Väli, M., Pärna, K., Tõnisson, M., Helander, A., McKee, M., & Leon, D.A. (2012). Alcohol and premature death in Estonian men: a study of forensic autopsies using novel biomarkers and proxy informants. *BMC Public Health*, *12,* 146.

Tuusov, J., Lang, K., Väli, M., Pärna, K., Tõnisson, M., Ringmets, I., McKee, M., Helander, A., & Leon, D.A. (2014). Prevalence of alcohol-related pathologies at autopsy: Estonian Forensic Study of Alcohol and Premature Death. *Addiction, 109*, 2018–26.

Tuusov, J., Vals, K., Tõnisson, M., Riikoja, A., Denissov, G., & Väli, M. (2012). Fatal poisoning in Estonia 2000–2009. Trends in illegal drug-related deaths. *Journal of Forensic and Legal Medicine, 20,* 51–6.

Törő, K., Väli, M., Lepik, D., Tuusov, J., Dunay, G., Marcsa, B., Pauliukevicius, A., Raudys, R., & Caplinskiene, M. (2013). Characteristics of cardiovascular deaths in forensic medical cases in Budapest, Vilnius and Tallinn. *Journal of Forensic and Legal Medicine, 20,* 968–71.

Töro, K., Szilvia, F., György, D., Pauliukevicius, A., Caplinskiene, M., Raudys, R., Lepik, D., Tuusov, J., Väli, M. (2011), Fatal traffic injuries among children and adolescents in three cities (capital Budapest, Vilnius, and Tallinn). *Journal of Forensic Sciences, 56,* 617–20.

Väli, M., Tuusov, J., Lang, K. & Pärna, K. (2011). Child abuse and the external cause of death in Estonia. In: Duarte Nuno Vieira (Ed.). Forensic Medicine from old problems to new challenges. Croatia: InTech – Open Access Publisher, pp. 178−88.

Erialane areng

Osalenud rahvusvahelistel konverentsidel (BMLA 2010, ISALM 2014, IALM 2016) posterettekannetega ning suulise ettekandega (BMLA 2017). Alates 2017. aastast Lõuna-Eesti kohtuarstliku ekspertiisiosakonna juhataja. Aastal 2019 omandatud kutsestandard, 7 tase.

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

1. **Heidi-Ingrid Maaroos**. The natural course of gastric ulcer in connection with chronic gastritis and *Helicobacter pylori*. Tartu, 1991.
2. **Mihkel Zilmer**. Na-pump in normal and tumorous brain tissues: Structural, functional and tumorigenesis aspects. Tartu, 1991.
3. **Eero Vasar**. Role of cholecystokinin receptors in the regulation of behaviour and in the action of haloperidol and diazepam. Tartu, 1992.
4. **Tiina Talvik**. Hypoxic-ischaemic brain damage in neonates (clinical, biochemical and brain computed tomographical investigation). Tartu, 1992.
5. **Ants Peetsalu**. Vagotomy in duodenal ulcer disease: A study of gastric acidity, serum pepsinogen I, gastric mucosal histology and *Helicobacter pylori*. Tartu, 1992.
6. **Marika Mikelsaar**. Evaluation of the gastrointestinal microbial ecosystem in health and disease. Tartu, 1992.
7. **Hele Everaus**. Immuno-hormonal interactions in chronic lymphocytic leukaemia and multiple myeloma. Tartu, 1993.
8. **Ruth Mikelsaar**. Etiological factors of diseases in genetically consulted children and newborn screening: dissertation for the commencement of the degree of doctor of medical sciences. Tartu, 1993.
9. **Agu Tamm**. On metabolic action of intestinal microflora: clinical aspects. Tartu, 1993.
10. **Katrin Gross**. Multiple sclerosis in South-Estonia (epidemiological and computed tomographical investigations). Tartu, 1993.
11. **Oivi Uibo**. Childhood coeliac disease in Estonia: occurrence, screening, diagnosis and clinical characterization. Tartu, 1994.
12. **Viiu Tuulik**. The functional disorders of central nervous system of chemistry workers. Tartu, 1994.
13. **Margus Viigimaa**. Primary haemostasis, antiaggregative and anticoagulant treatment of acute myocardial infarction. Tartu, 1994.
14. **Rein Kolk**. Atrial versus ventricular pacing in patients with sick sinus syndrome. Tartu, 1994.
15. **Toomas Podar**. Incidence of childhood onset type 1 diabetes mellitus in Estonia. Tartu, 1994.
16. **Kiira Subi**. The laboratory surveillance of the acute respiratory viral infections in Estonia. Tartu, 1995.
17. **Irja Lutsar**. Infections of the central nervous system in children (epidemiologic, diagnostic and therapeutic aspects, long term outcome). Tartu, 1995.
18. **Aavo Lang**. The role of dopamine, 5-hydroxytryptamine, sigma and NMDA receptors in the action of antipsychotic drugs. Tartu, 1995.
19. **Andrus Arak**. Factors influencing the survival of patients after radical surgery for gastric cancer. Tartu, 1996.

20. **Tõnis Karki**. Quantitative composition of the human lactoflora and method for its examination. Tartu, 1996.
21. **Reet Mändar**. Vaginal microflora during pregnancy and its transmission to newborn. Tartu, 1996.
22. **Triin Remmel**. Primary biliary cirrhosis in Estonia: epidemiology, clinical characterization and prognostication of the course of the disease. Tartu, 1996.
23. **Toomas Kivastik**. Mechanisms of drug addiction: focus on positive reinforcing properties of morphine. Tartu, 1996.
24. **Paavo Pokk**. Stress due to sleep deprivation: focus on $GABA_A$ receptor-chloride ionophore complex. Tartu, 1996.
25. **Kristina Allikmets**. Renin system activity in essential hypertension. Associations with atherothrombogenic cardiovascular risk factors and with the efficacy of calcium antagonist treatment. Tartu, 1996.
26. **Triin Parik**. Oxidative stress in essential hypertension: Associations with metabolic disturbances and the effects of calcium antagonist treatment. Tartu, 1996.
27. **Svetlana Päi**. Factors promoting heterogeneity of the course of rheumatoid arthritis. Tartu, 1997.
28. **Maarike Sallo**. Studies on habitual physical activity and aerobic fitness in 4 to 10 years old children. Tartu, 1997.
29. **Paul Naaber**. *Clostridium difficile* infection and intestinal microbial ecology. Tartu, 1997.
30. **Rein Pähkla**. Studies in pinoline pharmacology. Tartu, 1997.
31. **Andrus Juhan Voitk**. Outpatient laparoscopic cholecystectomy. Tartu, 1997.
32. **Joel Starkopf**. Oxidative stress and ischaemia-reperfusion of the heart. Tartu, 1997.
33. **Janika Kõrv**. Incidence, case-fatality and outcome of stroke. Tartu, 1998.
34. **Ülla Linnamägi**. Changes in local cerebral blood flow and lipid peroxidation following lead exposure in experiment. Tartu, 1998.
35. **Ave Minajeva**. Sarcoplasmic reticulum function: comparison of atrial and ventricular myocardium. Tartu, 1998.
36. **Oleg Milenin**. Reconstruction of cervical part of esophagus by revascularised ileal autografts in dogs. A new complex multistage method. Tartu, 1998.
37. **Sergei Pakriev**. Prevalence of depression, harmful use of alcohol and alcohol dependence among rural population in Udmurtia. Tartu, 1998.
38. **Allen Kaasik**. Thyroid hormone control over β-adrenergic signalling system in rat atria. Tartu, 1998.
39. **Vallo Matto**. Pharmacological studies on anxiogenic and antiaggressive properties of antidepressants. Tartu, 1998.
40. **Maire Vasar**. Allergic diseases and bronchial hyperreactivity in Estonian children in relation to environmental influences. Tartu, 1998.
41. **Kaja Julge**. Humoral immune responses to allergens in early childhood. Tartu, 1998.

42. **Heli Grünberg**. The cardiovascular risk of Estonian schoolchildren. A cross-sectional study of 9-, 12- and 15-year-old children. Tartu, 1998.
43. **Epp Sepp**. Formation of intestinal microbial ecosystem in children. Tartu, 1998.
44. **Mai Ots**. Characteristics of the progression of human and experimental glomerulopathies. Tartu, 1998.
45. **Tiina Ristimäe**. Heart rate variability in patients with coronary artery disease. Tartu, 1998.
46. **Leho Kõiv**. Reaction of the sympatho-adrenal and hypothalamo-pituitary-adrenocortical system in the acute stage of head injury. Tartu, 1998.
47. **Bela Adojaan**. Immune and genetic factors of childhood onset IDDM in Estonia. An epidemiological study. Tartu, 1999.
48. **Jakov Shlik**. Psychophysiological effects of cholecystokinin in humans. Tartu, 1999.
49. **Kai Kisand**. Autoantibodies against dehydrogenases of α-ketoacids. Tartu, 1999.
50. **Toomas Marandi**. Drug treatment of depression in Estonia. Tartu, 1999.
51. **Ants Kask**. Behavioural studies on neuropeptide Y. Tartu, 1999.
52. **Ello-Rahel Karelson**. Modulation of adenylate cyclase activity in the rat hippocampus by neuropeptide galanin and its chimeric analogs. Tartu, 1999.
53. **Tanel Laisaar**. Treatment of pleural empyema — special reference to intrapleural therapy with streptokinase and surgical treatment modalities. Tartu, 1999.
54. **Eve Pihl**. Cardiovascular risk factors in middle-aged former athletes. Tartu, 1999.
55. **Katrin Õunap**. Phenylketonuria in Estonia: incidence, newborn screening, diagnosis, clinical characterization and genotype/phenotype correlation. Tartu, 1999.
56. **Siiri Kõljalg**. *Acinetobacter* – an important nosocomial pathogen. Tartu, 1999.
57. **Helle Karro**. Reproductive health and pregnancy outcome in Estonia: association with different factors. Tartu, 1999.
58. **Heili Varendi**. Behavioral effects observed in human newborns during exposure to naturally occurring odors. Tartu, 1999.
59. **Anneli Beilmann**. Epidemiology of epilepsy in children and adolescents in Estonia. Prevalence, incidence, and clinical characteristics. Tartu, 1999.
60. **Vallo Volke**. Pharmacological and biochemical studies on nitric oxide in the regulation of behaviour. Tartu, 1999.
61. **Pilvi Ilves**. Hypoxic-ischaemic encephalopathy in asphyxiated term infants. A prospective clinical, biochemical, ultrasonographical study. Tartu, 1999.
62. **Anti Kalda**. Oxygen-glucose deprivation-induced neuronal death and its pharmacological prevention in cerebellar granule cells. Tartu, 1999.
63. **Eve-Irene Lepist**. Oral peptide prodrugs – studies on stability and absorption. Tartu, 2000.

64. **Jana Kivastik**. Lung function in Estonian schoolchildren: relationship with anthropometric indices and respiratory symptomas, reference values for dynamic spirometry. Tartu, 2000.
65. **Karin Kull**. Inflammatory bowel disease: an immunogenetic study. Tartu, 2000.
66. **Kaire Innos**. Epidemiological resources in Estonia: data sources, their quality and feasibility of cohort studies. Tartu, 2000.
67. **Tamara Vorobjova**. Immune response to *Helicobacter pylori* and its association with dynamics of chronic gastritis and epithelial cell turnover in antrum and corpus. Tartu, 2001.
68. **Ruth Kalda**. Structure and outcome of family practice quality in the changing health care system of Estonia. Tartu, 2001.
69. **Annika Krüüner**. *Mycobacterium tuberculosis* – spread and drug resistance in Estonia. Tartu, 2001.
70. **Marlit Veldi**. Obstructive Sleep Apnoea: Computerized Endopharyngeal Myotonometry of the Soft Palate and Lingual Musculature. Tartu, 2001.
71. **Anneli Uusküla**. Epidemiology of sexually transmitted diseases in Estonia in 1990–2000. Tartu, 2001.
72. **Ade Kallas**. Characterization of antibodies to coagulation factor VIII. Tartu, 2002.
73. **Heidi Annuk**. Selection of medicinal plants and intestinal lactobacilli as antimicrobil components for functional foods. Tartu, 2002.
74. **Aet Lukmann**. Early rehabilitation of patients with ischaemic heart disease after surgical revascularization of the myocardium: assessment of health-related quality of life, cardiopulmonary reserve and oxidative stress. A clinical study. Tartu, 2002.
75. **Maigi Eisen**. Pathogenesis of Contact Dermatitis: participation of Oxidative Stress. A clinical – biochemical study. Tartu, 2002.
76. **Piret Hussar**. Histology of the post-traumatic bone repair in rats. Elaboration and use of a new standardized experimental model – bicortical perforation of tibia compared to internal fracture and resection osteotomy. Tartu, 2002.
77. **Tõnu Rätsep**. Aneurysmal subarachnoid haemorrhage: Noninvasive monitoring of cerebral haemodynamics. Tartu, 2002.
78. **Marju Herodes**. Quality of life of people with epilepsy in Estonia. Tartu, 2003.
79. **Katre Maasalu**. Changes in bone quality due to age and genetic disorders and their clinical expressions in Estonia. Tartu, 2003.
80. **Toomas Sillakivi**. Perforated peptic ulcer in Estonia: epidemiology, risk factors and relations with *Helicobacter pylori*. Tartu, 2003.
81. **Leena Puksa**. Late responses in motor nerve conduction studies. F and A waves in normal subjects and patients with neuropathies. Tartu, 2003.
82. **Krista Lõivukene**. *Helicobacter pylori* in gastric microbial ecology and its antimicrobial susceptibility pattern. Tartu, 2003.

83. **Helgi Kolk**. Dyspepsia and *Helicobacter pylori* infection: the diagnostic value of symptoms, treatment and follow-up of patients referred for upper gastrointestinal endoscopy by family physicians. Tartu, 2003.
84. **Helena Soomer**. Validation of identification and age estimation methods in forensic odontology. Tartu, 2003.
85. **Kersti Oselin**. Studies on the human MDR1, MRP1, and MRP2 ABC transporters: functional relevance of the genetic polymorphisms in the *MDR1* and *MRP1* gene. Tartu, 2003.
86. **Jaan Soplepmann**. Peptic ulcer haemorrhage in Estonia: epidemiology, prognostic factors, treatment and outcome. Tartu, 2003.
87. **Margot Peetsalu**. Long-term follow-up after vagotomy in duodenal ulcer disease: recurrent ulcer, changes in the function, morphology and *Helicobacter pylori* colonisation of the gastric mucosa. Tartu, 2003.
88. **Kersti Klaamas**. Humoral immune response to *Helicobacter pylori* a study of host-dependent and microbial factors. Tartu, 2003.
89. **Pille Taba**. Epidemiology of Parkinson's disease in Tartu, Estonia. Prevalence, incidence, clinical characteristics, and pharmacoepidemiology. Tartu, 2003.
90. **Alar Veraksitš**. Characterization of behavioural and biochemical phenotype of cholecystokinin-2 receptor deficient mice: changes in the function of the dopamine and endopioidergic system. Tartu, 2003.
91. **Ingrid Kalev**. CC-chemokine receptor 5 (CCR5) gene polymorphism in Estonians and in patients with Type I and Type II diabetes mellitus. Tartu, 2003.
92. **Lumme Kadaja**. Molecular approach to the regulation of mitochondrial function in oxidative muscle cells. Tartu, 2003.
93. **Aive Liigant**. Epidemiology of primary central nervous system tumours in Estonia from 1986 to 1996. Clinical characteristics, incidence, survival and prognostic factors. Tartu, 2004.
94. **Andres, Kulla**. Molecular characteristics of mesenchymal stroma in human astrocytic gliomas. Tartu, 2004.
95. **Mari Järvelaid**. Health damaging risk behaviours in adolescence. Tartu, 2004.
96. **Ülle Pechter**. Progression prevention strategies in chronic renal failure and hypertension. An experimental and clinical study. Tartu, 2004.
97. **Gunnar Tasa**. Polymorphic glutathione S-transferases – biology and role in modifying genetic susceptibility to senile cataract and primary open angle glaucoma. Tartu, 2004.
98. **Tuuli Käämbre**. Intracellular energetic unit: structural and functional aspects. Tartu, 2004.
99. **Vitali Vassiljev**. Influence of nitric oxide syntase inhibitors on the effects of ethanol after acute and chronic ethanol administration and withdrawal. Tartu, 2004.

100. **Aune Rehema**. Assessment of nonhaem ferrous iron and glutathione redox ratio as markers of pathogeneticity of oxidative stress in different clinical groups. Tartu, 2004.
101. **Evelin Seppet**. Interaction of mitochondria and ATPases in oxidative muscle cells in normal and pathological conditions. Tartu, 2004.
102. **Eduard Maron**. Serotonin function in panic disorder: from clinical experiments to brain imaging and genetics. Tartu, 2004.
103. **Marje Oona**. *Helicobacter pylori* infection in children: epidemiological and therapeutic aspects. Tartu, 2004.
104. **Kersti Kokk**. Regulation of active and passive molecular transport in the testis. Tartu, 2005.
105. **Vladimir Järv**. Cross-sectional imaging for pretreatment evaluation and follow-up of pelvic malignant tumours. Tartu, 2005.
106. **Andre Õun**. Epidemiology of adult epilepsy in Tartu, Estonia. Incidence, prevalence and medical treatment. Tartu, 2005.
107. **Piibe Muda**. Homocysteine and hypertension: associations between homocysteine and essential hypertension in treated and untreated hypertensive patients with and without coronary artery disease. Tartu, 2005.
108. **Külli Kingo**. The interleukin-10 family cytokines gene polymorphisms in plaque psoriasis. Tartu, 2005.
109. **Mati Merila**. Anatomy and clinical relevance of the glenohumeral joint capsule and ligaments. Tartu, 2005.
110. **Epp Songisepp**. Evaluation of technological and functional properties of the new probiotic *Lactobacillus fermentum* ME-3. Tartu, 2005.
111. **Tiia Ainla**. Acute myocardial infarction in Estonia: clinical characteristics, management and outcome. Tartu, 2005.
112. **Andres Sell**. Determining the minimum local anaesthetic requirements for hip replacement surgery under spinal anaesthesia – a study employing a spinal catheter. Tartu, 2005.
113. **Tiia Tamme**. Epidemiology of odontogenic tumours in Estonia. Pathogenesis and clinical behaviour of ameloblastoma. Tartu, 2005.
114. **Triine Annus**. Allergy in Estonian schoolchildren: time trends and characteristics. Tartu, 2005.
115. **Tiia Voor**. Microorganisms in infancy and development of allergy: comparison of Estonian and Swedish children. Tartu, 2005.
116. **Priit Kasenõmm**. Indicators for tonsillectomy in adults with recurrent tonsillitis – clinical, microbiological and pathomorphological investigations. Tartu, 2005.
117. **Eva Zusinaite**. Hepatitis C virus: genotype identification and interactions between viral proteases. Tartu, 2005.
118. **Piret Kõll**. Oral lactoflora in chronic periodontitis and periodontal health. Tartu, 2006.
119. **Tiina Stelmach**. Epidemiology of cerebral palsy and unfavourable neurodevelopmental outcome in child population of Tartu city and county, Estonia Prevalence, clinical features and risk factors. Tartu, 2006.

120. **Katrin Pudersell**. Tropane alkaloid production and riboflavine excretion in the field and tissue cultures of henbane (*Hyoscyamus niger* L.). Tartu, 2006.
121. **Külli Jaako**. Studies on the role of neurogenesis in brain plasticity. Tartu, 2006.
122. **Aare Märtson**. Lower limb lengthening: experimental studies of bone regeneration and long-term clinical results. Tartu, 2006.
123. **Heli Tähepõld**. Patient consultation in family medicine. Tartu, 2006.
124. **Stanislav Liskmann**. Peri-implant disease: pathogenesis, diagnosis and treatment in view of both inflammation and oxidative stress profiling. Tartu, 2006.
125. **Ruth Rudissaar**. Neuropharmacology of atypical antipsychotics and an animal model of psychosis. Tartu, 2006.
126. **Helena Andreson**. Diversity of *Helicobacter pylori* genotypes in Estonian patients with chronic inflammatory gastric diseases. Tartu, 2006.
127. **Katrin Pruus**. Mechanism of action of antidepressants: aspects of serotoninergic system and its interaction with glutamate. Tartu, 2006.
128. **Priit Põder**. Clinical and experimental investigation: relationship of ischaemia/reperfusion injury with oxidative stress in abdominal aortic aneurysm repair and in extracranial brain artery endarterectomy and possibilities of protection against ischaemia using a glutathione analogue in a rat model of global brain ischaemia. Tartu, 2006.
129. **Marika Tammaru**. Patient-reported outcome measurement in rheumatoid arthritis. Tartu, 2006.
130. **Tiia Reimand**. Down syndrome in Estonia. Tartu, 2006.
131. **Diva Eensoo**. Risk-taking in traffic and Markers of Risk-Taking Behaviour in Schoolchildren and Car Drivers. Tartu, 2007.
132. **Riina Vibo**. The third stroke registry in Tartu, Estonia from 2001 to 2003: incidence, case-fatality, risk factors and long-term outcome. Tartu, 2007.
133. **Chris Pruunsild**. Juvenile idiopathic arthritis in children in Estonia. Tartu, 2007.
134. **Eve Õiglane-Šlik**. Angelman and Prader-Willi syndromes in Estonia. Tartu, 2007.
135. **Kadri Haller**. Antibodies to follicle stimulating hormone. Significance in female infertility. Tartu, 2007.
136. **Pille Ööpik**. Management of depression in family medicine. Tartu, 2007.
137. **Jaak Kals**. Endothelial function and arterial stiffness in patients with atherosclerosis and in healthy subjects. Tartu, 2007.
138. **Priit Kampus**. Impact of inflammation, oxidative stress and age on arterial stiffness and carotid artery intima-media thickness. Tartu, 2007.
139. **Margus Punab**. Male fertility and its risk factors in Estonia. Tartu, 2007.
140. **Alar Toom**. Heterotopic ossification after total hip arthroplasty: clinical and pathogenetic investigation. Tartu, 2007.

141. **Lea Pehme**. Epidemiology of tuberculosis in Estonia 1991–2003 with special regard to extrapulmonary tuberculosis and delay in diagnosis of pulmonary tuberculosis. Tartu, 2007.
142. **Juri Karjagin**. The pharmacokinetics of metronidazole and meropenem in septic shock. Tartu, 2007.
143. **Inga Talvik**. Inflicted traumatic brain injury shaken baby syndrome in Estonia – epidemiology and outcome. Tartu, 2007.
144. **Tarvo Rajasalu**. Autoimmune diabetes: an immunological study of type 1 diabetes in humans and in a model of experimental diabetes (in RIP-B7.1 mice). Tartu, 2007.
145. **Inga Karu**. Ischaemia-reperfusion injury of the heart during coronary surgery: a clinical study investigating the effect of hyperoxia. Tartu, 2007.
146. **Peeter Padrik**. Renal cell carcinoma: Changes in natural history and treatment of metastatic disease. Tartu, 2007.
147. **Neve Vendt**. Iron deficiency and iron deficiency anaemia in infants aged 9 to 12 months in Estonia. Tartu, 2008.
148. **Lenne-Triin Heidmets**. The effects of neurotoxins on brain plasticity: focus on neural Cell Adhesion Molecule. Tartu, 2008.
149. **Paul Korrovits**. Asymptomatic inflammatory prostatitis: prevalence, etiological factors, diagnostic tools. Tartu, 2008.
150. **Annika Reintam**. Gastrointestinal failure in intensive care patients. Tartu, 2008.
151. **Kristiina Roots**. Cationic regulation of Na-pump in the normal, Alzheimer's and CCK_2 receptor-deficient brain. Tartu, 2008.
152. **Helen Puusepp**. The genetic causes of mental retardation in Estonia: fragile X syndrome and creatine transporter defect. Tartu, 2009.
153. **Kristiina Rull**. Human chorionic gonadotropin beta genes and recurrent miscarriage: expression and variation study. Tartu, 2009.
154. **Margus Eimre**. Organization of energy transfer and feedback regulation in oxidative muscle cells. Tartu, 2009.
155. **Maire Link**. Transcription factors FoxP3 and AIRE: autoantibody associations. Tartu, 2009.
156. **Kai Haldre**. Sexual health and behaviour of young women in Estonia. Tartu, 2009.
157. **Kaur Liivak**. Classical form of congenital adrenal hyperplasia due to 21-hydroxylase deficiency in Estonia: incidence, genotype and phenotype with special attention to short-term growth and 24-hour blood pressure. Tartu, 2009.
158. **Kersti Ehrlich**. Antioxidative glutathione analogues (UPF peptides) – molecular design, structure-activity relationships and testing the protective properties. Tartu, 2009.
159. **Anneli Rätsep**. Type 2 diabetes care in family medicine. Tartu, 2009.
160. **Silver Türk**. Etiopathogenetic aspects of chronic prostatitis: role of mycoplasmas, coryneform bacteria and oxidative stress. Tartu, 2009.

161. **Kaire Heilman**. Risk markers for cardiovascular disease and low bone mineral density in children with type 1 diabetes. Tartu, 2009.
162. **Kristi Rüütel**. HIV-epidemic in Estonia: injecting drug use and quality of life of people living with HIV. Tartu, 2009.
163. **Triin Eller**. Immune markers in major depression and in antidepressive treatment. Tartu, 2009.
164. **Siim Suutre**. The role of TGF-β isoforms and osteoprogenitor cells in the pathogenesis of heterotopic ossification. An experimental and clinical study of hip arthroplasty. Tartu, 2010.
165. **Kai Kliiman**. Highly drug-resistant tuberculosis in Estonia: Risk factors and predictors of poor treatment outcome. Tartu, 2010.
166. **Inga Villa**. Cardiovascular health-related nutrition, physical activity and fitness in Estonia. Tartu, 2010.
167. **Tõnis Org**. Molecular function of the first PHD finger domain of Autoimmune Regulator protein. Tartu, 2010.
168. **Tuuli Metsvaht**. Optimal antibacterial therapy of neonates at risk of early onset sepsis. Tartu, 2010.
169. **Jaanus Kahu**. Kidney transplantation: Studies on donor risk factors and mycophenolate mofetil. Tartu, 2010.
170. **Koit Reimand**. Autoimmunity in reproductive failure: A study on associated autoantibodies and autoantigens. Tartu, 2010.
171. **Mart Kull**. Impact of vitamin D and hypolactasia on bone mineral density: a population based study in Estonia. Tartu, 2010.
172. **Rael Laugesaar**. Stroke in children – epidemiology and risk factors. Tartu, 2010.
173. **Mark Braschinsky**. Epidemiology and quality of life issues of hereditary spastic paraplegia in Estonia and implemention of genetic analysis in everyday neurologic practice. Tartu, 2010.
174. **Kadri Suija**. Major depression in family medicine: associated factors, recurrence and possible intervention. Tartu, 2010.
175. **Jarno Habicht**. Health care utilisation in Estonia: socioeconomic determinants and financial burden of out-of-pocket payments. Tartu, 2010.
176. **Kristi Abram**. The prevalence and risk factors of rosacea. Subjective disease perception of rosacea patients. Tartu, 2010.
177. **Malle Kuum**. Mitochondrial and endoplasmic reticulum cation fluxes: Novel roles in cellular physiology. Tartu, 2010.
178. **Rita Teek**. The genetic causes of early onset hearing loss in Estonian children. Tartu, 2010.
179. **Daisy Volmer**. The development of community pharmacy services in Estonia – public and professional perceptions 1993–2006. Tartu, 2010.
180. **Jelena Lissitsina**. Cytogenetic causes in male infertility. Tartu, 2011.
181. **Delia Lepik**. Comparison of gunshot injuries caused from Tokarev, Makarov and Glock 19 pistols at different firing distances. Tartu, 2011.
182. **Ene-Renate Pähkla**. Factors related to the efficiency of treatment of advanced periodontitis. Tartu, 2011.

183. **Maarja Krass**. L-Arginine pathways and antidepressant action. Tartu, 2011.
184. **Taavi Lai**. Population health measures to support evidence-based health policy in Estonia. Tartu, 2011.
185. **Tiit Salum**. Similarity and difference of temperature-dependence of the brain sodium pump in normal, different neuropathological, and aberrant conditions and its possible reasons. Tartu, 2011.
186. **Tõnu Vooder**. Molecular differences and similarities between histological subtypes of non-small cell lung cancer. Tartu, 2011.
187. **Jelena Štšepetova**. The characterisation of intestinal lactic acid bacteria using bacteriological, biochemical and molecular approaches. Tartu, 2011.
188. **Radko Avi**. Natural polymorphisms and transmitted drug resistance in Estonian HIV-1 CRF06_cpx and its recombinant viruses. Tartu, 2011, 116 p.
189. **Edward Laane**. Multiparameter flow cytometry in haematological malignancies. Tartu, 2011, 152 p.
190. **Triin Jagomägi**. A study of the genetic etiology of nonsyndromic cleft lip and palate. Tartu, 2011, 158 p.
191. **Ivo Laidmäe**. Fibrin glue of fish (*Salmo salar*) origin: immunological study and development of new pharmaceutical preparation. Tartu, 2012, 150 p.
192. **Ülle Parm**. Early mucosal colonisation and its role in prediction of invasive infection in neonates at risk of early onset sepsis. Tartu, 2012, 168 p.
193. **Kaupo Teesalu**. Autoantibodies against desmin and transglutaminase 2 in celiac disease: diagnostic and functional significance. Tartu, 2012, 142 p.
194. **Maksim Zagura**. Biochemical, functional and structural profiling of arterial damage in atherosclerosis. Tartu, 2012, 162 p.
195. **Vivian Kont**. Autoimmune regulator: characterization of thymic gene regulation and promoter methylation. Tartu, 2012, 134 p.
196. **Pirje Hütt**. Functional properties, persistence, safety and efficacy of potential probiotic lactobacilli. Tartu, 2012, 246 p.
197. **Innar Tõru**. Serotonergic modulation of CCK-4- induced panic. Tartu, 2012, 132 p.
198. **Sigrid Vorobjov**. Drug use, related risk behaviour and harm reduction interventions utilization among injecting drug users in Estonia: implications for drug policy. Tartu, 2012, 120 p.
199. **Martin Serg**. Therapeutic aspects of central haemodynamics, arterial stiffness and oxidative stress in hypertension. Tartu, 2012, 156 p.
200. **Jaanika Kumm**. Molecular markers of articular tissues in early knee osteoarthritis: a population-based longitudinal study in middle-aged subjects. Tartu, 2012, 159 p.
201. **Kertu Rünkorg**. Functional changes of dopamine, endopioid and endocannabinoid systems in CCK2 receptor deficient mice. Tartu, 2012, 125 p.
202. **Mai Blöndal**. Changes in the baseline characteristics, management and outcomes of acute myocardial infarction in Estonia. Tartu, 2012, 127 p.

203. **Jana Lass**. Epidemiological and clinical aspects of medicines use in children in Estonia. Tartu, 2012, 170 p.
204. **Kai Truusalu**. Probiotic lactobacilli in experimental persistent *Salmonella* infection. Tartu, 2013, 139 p.
205. **Oksana Jagur**. Temporomandibular joint diagnostic imaging in relation to pain and bone characteristics. Long-term results of arthroscopic treatment. Tartu, 2013, 126 p.
206. **Katrin Sikk**. Manganese-ephedrone intoxication – pathogenesis of neurological damage and clinical symptomatology. Tartu, 2013, 125 p.
207. **Kai Blöndal**. Tuberculosis in Estonia with special emphasis on drug-resistant tuberculosis: Notification rate, disease recurrence and mortality. Tartu, 2013, 151 p.
208. **Marju Puurand**. Oxidative phosphorylation in different diseases of gastric mucosa. Tartu, 2013, 123 p.
209. **Aili Tagoma**. Immune activation in female infertility: Significance of autoantibodies and inflammatory mediators. Tartu, 2013, 135 p.
210. **Liis Sabre**. Epidemiology of traumatic spinal cord injury in Estonia. Brain activation in the acute phase of traumatic spinal cord injury. Tartu, 2013, 135 p.
211. **Merit Lamp**. Genetic susceptibility factors in endometriosis. Tartu, 2013, 125 p.
212. **Erik Salum**. Beneficial effects of vitamin D and angiotensin II receptor blocker on arterial damage. Tartu, 2013, 167 p.
213. **Maire Karelson**. Vitiligo: clinical aspects, quality of life and the role of melanocortin system in pathogenesis. Tartu, 2013, 153 p.
214. **Kuldar Kaljurand**. Prevalence of exfoliation syndrome in Estonia and its clinical significance. Tartu, 2013, 113 p.
215. **Raido Paasma**. Clinical study of methanol poisoning: handling large outbreaks, treatment with antidotes, and long-term outcomes. Tartu, 2013, 96 p.
216. **Anne Kleinberg**. Major depression in Estonia: prevalence, associated factors, and use of health services. Tartu, 2013, 129 p.
217. **Triin Eglit**. Obesity, impaired glucose regulation, metabolic syndrome and their associations with high-molecular-weight adiponectin levels. Tartu, 2014, 115 p.
218. **Kristo Ausmees**. Reproductive function in middle-aged males: Associations with prostate, lifestyle and couple infertility status. Tartu, 2014, 125 p.
219. **Kristi Huik**. The influence of host genetic factors on the susceptibility to HIV and HCV infections among intravenous drug users. Tartu, 2014, 144 p.
220. **Liina Tserel**. Epigenetic profiles of monocytes, monocyte-derived macrophages and dendritic cells. Tartu, 2014, 143 p.
221. **Irina Kerna**. The contribution of *ADAM12* and *CILP* genes to the development of knee osteoarthritis. Tartu, 2014, 152 p.

222. **Ingrid Liiv**. Autoimmune regulator protein interaction with DNA-dependent protein kinase and its role in apoptosis. Tartu, 2014, 143 p.
223. **Liivi Maddison**. Tissue perfusion and metabolism during intra-abdominal hypertension. Tartu, 2014, 103 p.
224. **Krista Ress**. Childhood coeliac disease in Estonia, prevalence in atopic dermatitis and immunological characterisation of coexistence. Tartu, 2014, 124 p.
225. **Kai Muru**. Prenatal screening strategies, long-term outcome of children with marked changes in maternal screening tests and the most common syndromic heart anomalies in Estonia. Tartu, 2014, 189 p.
226. **Kaja Rahu**. Morbidity and mortality among Baltic Chernobyl cleanup workers: a register-based cohort study. Tartu, 2014, 155 p.
227. **Klari Noormets**. The development of diabetes mellitus, fertility and energy metabolism disturbances in a Wfs1-deficient mouse model of Wolfram syndrome. Tartu, 2014, 132 p.
228. **Liis Toome**. Very low gestational age infants in Estonia. Tartu, 2014, 183 p.
229. **Ceith Nikkolo**. Impact of different mesh parameters on chronic pain and foreign body feeling after open inguinal hernia repair. Tartu, 2014, 132 p.
230. **Vadim Brjalin**. Chronic hepatitis C: predictors of treatment response in Estonian patients. Tartu, 2014, 122 p.
231. **Vahur Metsna**. Anterior knee pain in patients following total knee arthroplasty: the prevalence, correlation with patellar cartilage impairment and aspects of patellofemoral congruence. Tartu, 2014, 130 p.
232. **Marju Kase**. Glioblastoma multiforme: possibilities to improve treatment efficacy. Tartu, 2015, 137 p.
233. **Riina Runnel**. Oral health among elementary school children and the effects of polyol candies on the prevention of dental caries. Tartu, 2015, 112 p.
234. **Made Laanpere**. Factors influencing women's sexual health and reproductive choices in Estonia. Tartu, 2015, 176 p.
235. **Andres Lust**. Water mediated solid state transformations of a polymorphic drug – effect on pharmaceutical product performance. Tartu, 2015, 134 p.
236. **Anna Klugman**. Functionality related characterization of pretreated wood lignin, cellulose and polyvinylpyrrolidone for pharmaceutical applications. Tartu, 2015, 156 p.
237. **Triin Laisk-Podar**. Genetic variation as a modulator of susceptibility to female infertility and a source for potential biomarkers. Tartu, 2015, 155 p.
238. **Mailis Tõnisson**. Clinical picture and biochemical changes in blood in children with acute alcohol intoxication. Tartu, 2015, 100 p.
239. **Kadri Tamme**. High volume haemodiafiltration in treatment of severe sepsis – impact on pharmacokinetics of antibiotics and inflammatory response. Tartu, 2015, 133 p.

240. **Kai Part**. Sexual health of young people in Estonia in a social context: the role of school-based sexuality education and youth-friendly counseling services. Tartu, 2015, 203 p.
241. **Urve Paaver**. New perspectives for the amorphization and physical stabilization of poorly water-soluble drugs and understanding their dissolution behavior. Tartu, 2015, 139 p.
242. **Aleksandr Peet**. Intrauterine and postnatal growth in children with HLA-conferred susceptibility to type 1 diabetes. Tartu. 2015, 146 p.
243. **Piret Mitt**. Healthcare-associated infections in Estonia – epidemiology and surveillance of bloodstream and surgical site infections. Tartu, 2015, 145 p.
244. **Merli Saare**. Molecular Profiling of Endometriotic Lesions and Endometria of Endometriosis Patients. Tartu, 2016, 129 p.
245. **Kaja-Triin Laisaar**. People living with HIV in Estonia: Engagement in medical care and methods of increasing adherence to antiretroviral therapy and safe sexual behavior. Tartu, 2016, 132 p.
246. **Eero Merilind**. Primary health care performance: impact of payment and practice-based characteristics. Tartu, 2016, 120 p.
247. **Jaanika Kärner**. Cytokine-specific autoantibodies in AIRE deficiency. Tartu, 2016, 182 p.
248. **Kaido Paapstel**. Metabolomic profile of arterial stiffness and early biomarkers of renal damage in atherosclerosis. Tartu, 2016, 173 p.
249. **Liidia Kiisk**. Long-term nutritional study: anthropometrical and clinico-laboratory assessments in renal replacement therapy patients after intensive nutritional counselling. Tartu, 2016, 207 p.
250. **Georgi Nellis**. The use of excipients in medicines administered to neonates in Europe. Tartu, 2017, 159 p.
251. **Aleksei Rakitin**. Metabolic effects of acute and chronic treatment with valproic acid in people with epilepsy. Tartu, 2017, 125 p.
252. **Eveli Kallas**. The influence of immunological markers to susceptibility to HIV, HBV, and HCV infections among persons who inject drugs. Tartu, 2017, 138 p.
253. **Tiina Freimann**. Musculoskeletal pain among nurses: prevalence, risk factors, and intervention. Tartu, 2017, 125 p.
254. **Evelyn Aaviksoo**. Sickness absence in Estonia: determinants and influence of the sick-pay cut reform. Tartu, 2017, 121 p.
255. **Kalev Nõupuu**. Autosomal-recessive Stargardt disease: phenotypic heterogeneity and genotype-phenotype associations. Tartu, 2017, 131 p.
256. **Ho Duy Binh**. Osteogenesis imperfecta in Vietnam. Tartu, 2017, 125 p.
257. **Uku Haljasorg**. Transcriptional mechanisms in thymic central tolerance. Tartu, 2017, 147 p.
258. **Živile Riispere**. IgA Nephropathy study according to the Oxford Classification: IgA Nephropathy clinical-morphological correlations, disease progression and the effect of renoprotective therapy. Tartu, 2017, 129 p.

259. **Hiie Soeorg**. Coagulase-negative staphylococci in gut of preterm neonates and in breast milk of their mothers. Tartu, 2017, 216 p.
260. **Anne-Mari Anton Willmore**. Silver nanoparticles for cancer research. Tartu, 2017, 132 p.
261. **Ott Laius**. Utilization of osteoporosis medicines, medication adherence and the trend in osteoporosis related hip fractures in Estonia. Tartu, 2017, 134 p.
262. **Alar Aab**. Insights into molecular mechanisms of asthma and atopic dermatitis. Tartu, 2017, 164 p.
263. **Sander Pajusalu**. Genome-wide diagnostics of Mendelian disorders: from chromosomal microarrays to next-generation sequencing. Tartu, 2017, 146 p.
264. **Mikk Jürisson**. Health and economic impact of hip fracture in Estonia. Tartu, 2017, 164 p.
265. **Kaspar Tootsi**. Cardiovascular and metabolomic profiling of osteoarthritis. Tartu, 2017, 150 p.
266. **Mario Saare**. The influence of AIRE on gene expression – studies of transcriptional regulatory mechanisms in cell culture systems. Tartu, 2017, 172 p.
267. **Piia Jõgi**. Epidemiological and clinical characteristics of pertussis in Estonia. Tartu, 2018, 168 p.
268. **Elle Põldoja**. Structure and blood supply of the superior part of the shoulder joint capsule. Tartu, 2018, 116 p.
269. **Minh Son Nguyen**. Oral health status and prevalence of temporomandibular disorders in 65–74-year-olds in Vietnam. Tartu, 2018, 182 p.
270. **Kristian Semjonov**. Development of pharmaceutical quench-cooled molten and melt-electrospun solid dispersions for poorly water-soluble indomethacin. Tartu, 2018, 125 p.
271. **Janne Tiigimäe-Saar**. Botulinum neurotoxin type A treatment for sialorrhea in central nervous system diseases. Tartu, 2018, 109 p.
272. **Veiko Vengerfeldt**. Apical periodontitis: prevalence and etiopathogenetic aspects. Tartu, 2018, 150 p.
273. **Rudolf Bichele**. TNF superfamily and AIRE at the crossroads of thymic differentiation and host protection against *Candida albicans* infection. Tartu, 2018, 153 p.
274. **Olga Tšuiko**. Unravelling Chromosomal Instability in Mammalian Preimplantation Embryos Using Single-Cell Genomics. Tartu, 2018, 169 p.
275. **Kärt Kriisa**. Profile of acylcarnitines, inflammation and oxidative stress in first-episode psychosis before and after antipsychotic treatment. Tartu, 2018, 145 p.
276. **Xuan Dung Ho**. Characterization of the genomic profile of osteosarcoma. Tartu, 2018, 144 p.
277. **Karit Reinson**. New Diagnostic Methods for Early Detection of Inborn Errors of Metabolism in Estonia. Tartu, 2018, 201 p.

278. **Mari-Anne Vals**. Congenital N-glycosylation Disorders in Estonia. Tartu, 2019, 148 p.
279. **Liis Kadastik-Eerme**. Parkinson's disease in Estonia: epidemiology, quality of life, clinical characteristics and pharmacotherapy. Tartu, 2019, 202 p.
280. **Hedi Hunt**. Precision targeting of intraperitoneal tumors with peptide-guided nanocarriers. Tartu, 2019, 179 p.
281. **Rando Porosk**. The role of oxidative stress in Wolfram syndrome 1 and hypothermia. Tartu, 2019, 123 p.
282. **Ene-Ly Jõgeda**. The influence of coinfections and host genetic factor on the susceptibility to HIV infection among people who inject drugs. Tartu, 2019, 126 p.
283. **Kristel Ehala-Aleksejev**. The associations between body composition, obesity and obesity-related health and lifestyle conditions with male reproductive function. Tartu, 2019, 138 p.
284. **Aigar Ottas**. The metabolomic profiling of psoriasis, atopic dermatitis and atherosclerosis. Tartu, 2019, 136 p.
285. **Elmira Gurbanova**. Specific characteristics of tuberculosis in low default, but high multidrug–resistance prison setting. Tartu, 2019, 129 p.
286. **Van Thai Nguyeni**. The first study of the treatment outcomes of patients with cleft lip and palate in Central Vietnam. Tartu, 2019, 144 p.
287. **Maria Yakoreva**. Imprinting Disorders in Estonia. Tartu, 2019, 187 p.
288. **Kadri Rekker**. The putative role of microRNAs in endometriosis pathogenesis and potential in diagnostics. Tartu, 2019, 140 p.
289. **Ülle Võhma**. Association between personality traits, clinical characteristics and pharmacological treatment response in panic disorder. Tartu, 2019, 121 p.
290. **Aet Saar**. Acute myocardial infarction in Estonia 2001–2014: towards risk-based prevention and management. Tartu, 2019, 124 p.
291. **Toomas Toomsoo**. Transcranial brain sonography in the Estonian cohort of Parkinson's disease. Tartu, 2019, 114 p.
292. **Lidiia Zhytnik**. Inter- and intrafamilial diversity based on genotype and phenotype correlations of Osteogenesis Imperfecta. Tartu, 2019, 224 p.
293. **Pilleriin Soodla**. Newly HIV-infected people in Estonia: estimation of incidence and transmitted drug resistance. Tartu, 2019, 194 p.
294. **Kristiina Ojamaa**. Epidemiology of gynecological cancer in Estonia. Tartu, 2020, 133 p.
295. **Marianne Saard**. Modern Cognitive and Social Intervention Techniques in Paediatric Neurorehabilitation for Children with Acquired Brain Injury. Tartu, 2020, 168 p.
296. **Julia Maslovskaja**. The importance of DNA binding and DNA breaks for AIRE-mediated transcriptional activation. Tartu, 2020, 162 p.
297. **Natalia Lobanovskaya**. The role of PSA-NCAM in the survival of retinal ganglion cells. Tartu, 2020, 105 p.

298. **Madis Rahu**. Structure and blood supply of the postero-superior part of the shoulder joint capsule with implementation of surgical treatment after anterior traumatic dislocation. Tartu, 2020, 104 p.
299. **Helen Zirnask**. Luteinizing hormone (LH) receptor expression in the penis and its possible role in pathogenesis of erectile disturbances. Tartu, 2020, 87 p.
300. **Kadri Toome**. Homing peptides for targeting of brain diseases. Tartu, 2020, 152 p.
301. **Maarja Hallik**. Pharmacokinetics and pharmacodynamics of inotropic drugs in neonates. Tartu, 2020, 172 p.
302. **Raili Müller**. Cardiometabolic risk profile and body composition in early rheumatoid arthritis. Tartu, 2020, 133 p.
303. **Sergo Kasvandik**. The role of proteomic changes in endometrial cells – from the perspective of fertility and endometriosis. Tartu, 2020, 191 p.
304. **Epp Kaleviste**. Genetic variants revealing the role of STAT1/STAT3 signaling cytokines in immune protection and pathology. Tartu, 2020, 189 p.
305. **Sten Saar**. Epidemiology of severe injuries in Estonia. Tartu, 2020, 104 p.
306. **Kati Braschinsky**. Epidemiology of primary headaches in Estonia and applicability of web-based solutions in headache epidemiology research. Tartu, 2020, 129 p.
307. **Helen Vaher**. MicroRNAs in the regulation of keratinocyte responses in *psoriasis vulgaris* and atopic dermatitis. Tartu, 2020, 242 p.
308. **Liisi Raam**. Molecular Alterations in the Pathogenesis of Two Chronic Dermatoses – Vitiligo and Psoriasis. Tartu, 2020, 164 p.
309. **Artur Vetkas**. Long-term quality of life, emotional health, and associated factors in patients after aneurysmal subarachnoid haemorrhage. Tartu, 2020, 127 p.
310. **Teele Kasepalu**. Effects of remote ischaemic preconditioning on organ damage and acylcarnitines' metabolism in vascular surgery. Tartu, 2020, 130 p.
311. **Prakash Lingasamy**. Development of multitargeted tumor penetrating peptides. Tartu, 2020, 246 p.
312. **Lille Kurvits.** Parkinson's disease as a multisystem disorder: whole transcriptome study in Parkinson's disease patients' skin and blood. Tartu, 2021, 142 p.
313. **Mariliis Põld.** Smoking, attitudes towards smoking behaviour, and nicotine dependence among physicians in Estonia: cross-sectional surveys 1982–2014. Tartu, 2021, 172 p.
314. **Triin Kikas**. Single nucleotide variants affecting placental gene expression and pregnancy outcome. Tartu, 2021, 160 p.
315. **Hedda Lippus-Metsaots**. Interpersonal violence in Estonia: prevalence, impact on health and health behaviour. Tartu, 2021, 172 p.

316. **Georgi Dzaparidze.** Quantification and evaluation of the diagnostic significance of adenocarcinoma-associated microenvironmental changes in the prostate using modern digital pathology solutions. Tartu, 2021, 132 p.
317. **Tuuli Sedman.** New avenues for GLP1 receptor agonists in the treatment of diabetes. Tartu, 2021, 118 p.
318. **Martin Padar.** Enteral nutrition, gastrointestinal dysfunction and intestinal biomarkers in critically ill patients. Tartu, 2021, 189 p.
319. **Siim Schneider.** Risk factors, etiology and long-term outcome in young ischemic stroke patients in Estonia. Tartu, 2021, 131 p.
320. **Konstantin Ridnõi.** Implementation and effectiveness of new prenatal diagnostic strategies in Estonia. Tartu, 2021, 191 p.
321. **Risto Vaikjärv.** Etiopathogenetic and clinical aspects of peritonsillar abscess. Tartu, 2021, 115 p.
322. **Liis Preem.** Design and characterization of antibacterial electrospun drug delivery systems for wound infections. Tartu, 2022, 220 p.
323. **Keerthie Dissanayake.** Preimplantation embryo-derived extracellular vesicles: potential as an embryo quality marker and their role during the embryo-maternal communication. Tartu, 2022, 203 p.
324. **Laura Viidik.** 3D printing in pharmaceutics: a new avenue for fabricating therapeutic drug delivery systems. Tartu, 2022, 139 p.
325. **Kasun Godakumara.** Extracellular vesicle mediated embryo-maternal communication – A tool for evaluating functional competency of preimplantation embryos. Tartu, 2022, 176 p.
326. **Hindrek Teder.** Developing computational methods and workflows for targeted and whole-genome sequencing based non-invasive prenatal testing. Tartu, 2022, 138 p.