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PROSPEKTIVNÍ STUDIE DLOUHODOBÝCH ZRAKOVÝCH NÁSLEDKŮ AKUTNÍCH INTOXIKACÍ METANOLEM

Prospective study of long-term visual sequelae of acute methanol poisonings

DISERTAČNÍ PRÁCE

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ABSTRAKT

Kontext: Otrava metanolem je život ohrožující stav, který způsobuje toxickou neuropatii zrakového nervu s možnými dlouhodobými zrakovými následky u osob přeživších otravu.

Cíl: Zjistit prevalenci, charakter, dynamiku a klíčové determinanty chronických morfologických a funkčních změn zrakového systému v průběhu 4 let po akutní optické neuropatii způsobené intoxikací metanolem.

Materiál a metodika: Celkem 55 pacientů s potvrzenou diagnózou otravy metanolem ve věku 46.7 ± 3.6 let (46 mužů a 9 žen) a 41 kontrol bylo vyšetřeno v rámci prospektivní longitudinální kohortové studie. Pacienti byli vyšetřeni 4.9 ± 0.6, 25.0 ± 0.6 a 49.9 ± 0.5 měsíců po propuštění z nemocnice. Protokol klinického vyšetření zahrnoval měření zrakových evokovaných potenciálů (VEP), optickou koherenční tomografii tloušťky vrstvy nervových vláken sítnice (RNFL), magnetické rezonanční vyšetření mozku (MRI), kompletní oftalmologické vyšetření, biochemická vyšetření a určení genotypu apolipoproteinu E (ApoE).

Výsledky: Ze 42/55 pacientů, kteří absolvovali všechna tři kola vyšetření, abnormální tloušťka RNFL byla zjištěna u 13 (31%) a progredující pokles tloušťky RNFL v době sledování byl pozorován u 10 (24%) pacientů. Riziko chronického poklesu globální RNFL pro pH arteriální krve <7.3 při příjmu bylo 11.65 (1.91-71.12; 95% CI) po adjustaci na věk a pohlaví. Progredující ztráta zrakových funkcí byla registrována u 7 z 10 pacientů s chronickou axonální degenerací sítnice. Abnormální latence vlny P1 evokovaného potenciálu byla registrována na 18/42 pravých očích (OD) a na 21/42 levých očích (OS), abnormální amplituda N1P1 byla pozorována na 10/42 OD a OS. Průměrné zkrácení latence vlny P1 v důsledku remyelinizace v době sledování bylo 15.0 ± 2.0 ms pro OD a 14.9 ± 2.4 ms pro OS. Další pokles amplitudy N1P1 ≥ 1.0 mcV v době sledování byl pozorován u 17 z 36 pacientů (47%) s měřitelnou amplitudou. Nositelé alely ApoE4 měli menší tloušťku globální a temporální RNFL a delší latenci vlny P1 v porovnání s nositeli jiných alel ApoE (všechna $p < 0.05$). Odds ratio pro abnormální funkce zrakového nervu bylo 8.92 (3.00 – 36.50; 95% CI) pro nositelé ApoE4 alely ($p < 0.001$). Pacienti s abnormální tloušťkou RNFL měli MRI známky poškození mozku v 10/13 případech versus 8/29 případů s normální tloušťkou RNFL ($p=0.003$). Byla přítomna signifikantní pozitivní asociace mezi přednemocničním podáním etanolu jako antidota pro první pomoc, rychlou korekcí acidemie a eliminací kyseliny mravenčí pomocí intermitentní hemodialýzy a lepším morfologickým nálezem na oční sítnici i funkčním stavem zrakového nervu (všechna $p < 0.05$).

Závěr: Optická neuropatie způsobená metanolem může vést k chronické ztrátě axonů oční sítnice v letech následujících po otravě. Vstupní pH arteriální krve je nejvýznamnějším prognostickým faktorem pro dynamiku změn tloušťky RNFL. Signifikantní asociace byla přítomna mezi chronickou neurodegenerací sítnice, progredující ztrátou zrakových funkcí a nekrotickými lézemi v mozku. Zlepšení konduktivity zrakového nervu bylo pozorováno u více než 80 % pacientů, avšak amplituda evokovaného potenciálu měla tendenci k dalšímu poklesu v průběhu 4 let sledování. Nositelé alely ApoE4 měli menší tloušťku RNFL, prodlouženou latenci vlny P1 a častější poškození mozku v důsledku otravy metanolem než pacienti – nositelé jiných alel ApoE. Přednemocniční podání etanolu a intermitentní hemodialýza měly pozitivní preventivní účinek z hlediska rizika dlouhodobých zrakových následků otrav metanolem.

Klíčová slova: akutní otrava metanolem, toxická optická neuropatie, zrakové následky, zrakové evokované potenciály, optická koherenční tomografie, tloušťka vrstvy nervových vláken sítnice.

ABSTRACT

Background: Methanol poisoning is a life-threatening condition which induces acute toxic optic neuropathy with possible long-term visual sequelae in survivors.

Aim: To study the prevalence, character, dynamics, and key determinants of chronic morphological and functional visual pathway changes during 4 years after methanol-induced optic neuropathy.

Methods: A total of 55 patients with confirmed methanol poisoning with mean age 46.7 ± 3.6 years (46 males and 9 females), and 41 controls were included in this prospective longitudinal cohort study. The patients were examined 4.9 ± 0.6, 25.0 ± 0.6, and 49.9 ± 0.5 months after discharge. The following tests were performed: visual evoked potential (VEP), optical coherence tomography with retinal nerve fiber layer (RNFL) measurement, brain magnetic resonance imaging (MRI), complete ocular examination, biochemical tests, and apolipoprotein E (ApoE) genotyping.

Results: Of 42/55 patients with all three consecutive examinations, abnormal RNFL thickness was registered in 13 (31%) and chronic axonal loss during the observation period was found in 10 (24%) patients. The risk estimate of chronic global RNFL loss for arterial blood pH<7.3 at admission was: 11.65 (1.91-71.12; 95% CI) after adjusting for age and sex. The patients with chronic axonal degeneration demonstrated further progressive visual loss in 7/10 cases. Abnormal VEP latency P1 was registered in 18/42 right eyes (OD) and 21/42 left eyes (OS), abnormal amplitude N1P1 in 10/42 OD and OS. Mean latency P1 shortening due to remyelination during the follow-up period was 15.0 ± 2.0 ms for OD and 14.9 ± 2.4 ms for OS. A further decrease of amplitude N1P1 ≥ 1.0 mcV was observed in 17 of 36 patients (47%) with measurable amplitude. ApoE4 allele carriers had lower global and temporal RNFL thickness and longer latency P1 compared to the non-carriers (all $p < 0.05$). The odds ratio for abnormal visual function was 8.92 (3.00 – 36.50; 95% CI) for ApoE4 allele carriers ($p < 0.001$). The patients with abnormal RNFL thickness had MRI signs of brain damage in 10/13 versus 8/29 cases with normal RNFL thickness ($p=0.003$). Pre-hospital ethanol administration as a “first aid” antidote and rapid acidemia correction and formic acid elimination by intermittent hemodialysis were associated with better visual outcome (both $p < 0.05$).

Conclusion: Methanol-induced toxic optic neuropathy may lead to chronic retinal axonal loss during the following years. Arterial blood pH on admission was the strongest predictor of chronic RNFL thickness decrease. Chronic retinal neurodegeneration was associated with progressive loss of visual functions and necrotic brain lesions. Improvement of optic nerve conductivity occurred in more than 80% of patients, but the amplitude of evoked potential tended to decrease during 4 years of observation. ApoE4 allele carriers demonstrated lower RNFL thickness, longer latency P1, and more frequent methanol-induced brain damage compared to the non-carriers. Pre-hospital ethanol administration as a “first aid” antidote and intermittent hemodialysis demonstrated positive preventive effect against long-term visual sequelae of methanol poisoning.

Key words: acute methanol poisoning, toxic optic neuropathy, visual sequelae, visual evoked potentials, optic coherence tomography, retinal nerve fiber layer.

O B S A H

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ZKRATKY

AACT – Americká akademie klinické toxikologie
ABG – arteriální plyny
ADH – alkoholdehydrogenáza
AG – aniontové okno
ALDH – aldehyddehydrogenáza
BD – deficit bází
CDT – karbohydrát deficientní transferin
CNS – centrální nervový systém
CT – počítačová tomografie
CVVH/HD/HDF – kontinuální veno-venózní hemofiltrace / hemodialýza/ hemodiafiltrace
et al.. – et al.ii – a jiní
EAPCCT – Evropská asociace toxikologických středisek a klinických toxikologů
EtOH - etanol
GI – gastrointestinální
GCS – Glasgow Coma Scale
ICU – jednotka intenzivní péče
IHD – intermitentní hemodialýza
MEOS – systém mikrosomální oxidace etanolu
MetOH – metanol
MRI – magnetické rezonanční vyšetření
OCT – optická koherenční tomografie
OG – osmolální okno
OR – odds ratio
RNFL – vrstva nervových vláken sítnice
ROS – reaktivní sloučeniny kyslíku
S – (například, S-methanol, S-formate) – koncentrace v krevním séru
SD – standardní odchylka
THF – tetrahydrofolát
TSH – thyreotropní hormon
VD – zrakové potíže
VEP – zrakové evokované potenciály
VS – zrakové následky otravy metanolem

1. ÚVOD

Metanol neboli metylalkohol, byl poprvé izolován v roce 1661 Robertem Boylem (Boyle, 1661). V průběhu následujících dvou století se objevovaly pouze ojedinělé případy otrav, které byly vysvětlovány přítomností velkého množství toxických příměsí v metanolu (Wood, 1906). V roce 1896 byl vynalezen účinný a levný způsob čištění metanolu, což vedlo k jeho širšímu využití jako „vynikající náhrady etanolu“ v různých obchodních přípravcích (toaletních potřebách, balzámech, extraktech, mastích atd.) a později i v alkoholických nápojích (Ziegler, 1921). Od etanolu se metanol neliší svým vzhledem, chutí ani vůní.

Již na začátku 20. století Buller a Wood popsali 314 případů intoxikace metylalkoholem ve Spojených Státech, včetně 158 případů slepoty v důsledku této otravy (Buller and Wood, 1904; Wood, 1905). V roce 1911 se v Německu během vánočního týdne v Berlíně přiotrávilo 163 lidí, z nichž 72 zemřeli (Pincus, 1912; Stadelmann & Magnus-Levy, 1912). Další případy otrav byly zaznamenány v Rusku, Polsku, Maďarsku i v jiných zemích. Dlouhou dobu ale chyběly důkazy o toxicitě metanolu, protože při pokusech na laboratorních zvířatech se neprojevovaly příznaky typické pro otravu lidí (Røe, 1946). Přestože již v roce 1923 Reif popsal klinický průběh otravy po požití chemicky čistého metanolu skupinou přístavních dělníků, pochybnosti o toxicitě metanolu přetrvávaly až do roku 1936 (Bennett, 1953; Reif, 1923). Později se podařilo v experimentech na vyšších primátech vyvolat stejný obraz otravy jako u člověka.

Nicméně i po prokázání toxicity metanolu často docházelo k hromadným otravám i v druhé polovině 20. století a to nejen v zemích třetího světa, ale i v rozvinutých zemích (Jacobsen et al., 1945; Kaplan & Levreault, 1945; Pronnie et al., 1946). Metanol bývá součástí některých nemrznoucích směsí, kapalin do ostřikovačů skel automobilů i jiných technických kapalin. K otravě metanolem může dojít, pokud se člověk omylem nebo záměrně napije tohoto technického metanolu nebo častěji metanolem pančovaného alkoholu (Benton et al., 1953;

Divekar et al., 1974; Naraqi et al., 1979; Sejersted et al., 1981; Tonkabony et al., 1975).

V současné době se většina hromadných otrav metanolem vyskytuje zejména v zemích třetího světa, kde se metanol se přidává do lihovin jako levná náhrada etanolu (Albertson, 1999). Zprávy o hromadných otravách přicházejí z Indie (Bade & Sapre,1981; Dilip et al., 2013; Mittal et al., 1991; Mohan et al., 2001; Ravichandran et al., 1984; Saxena, 1999; Shah et al., 2012), Bangladéše (Chowdhury et al., 2014), Turecka (Azmak, 2006; Duman et al., 2003; Gülmen et al., 2006; Kalkan et al., 2003; Karadeniz & Birincioglu, 2011; Unsal et al., 2012; Yaycia et al., 2003), Iránu (Hassanian-Moghaddam et al., 2015a; Massoumi et al., 2012; Moghadami et al., 2008), Indonésie (Giovanetti, 2013; Koehrer et al., 2011), Jordanu (Abdallat et al., 2009), Keni (Ahmad, 2000), Libye (Ben Taleb & Bahelah, 2014), Tuniska (Brahmi et al., 2007), Turecka (Adanir et al., 2005), Súdánu (Abdul Rahim & Al Shiekh, 2012), a dalších zemí Asie a Afriky.

Během let 2000-2012 se ve světě odehrálo více než 50 hromadných otrav metanolem, postiženo bylo více než 5000 osob a nejméně 2000 lidí zemřelo (Zhang et al., 2015). Mezi nedávné hromadné otravy v zemích Evropské unie patří otrava v Estonsku v roce 2001 s více než 150 případy (Paasma et al., 2007; Paasma, 2013), v Norsku v roce 2002 s více než 50 případy (Hovda et al., 2005a) a v České republice v roce 2012 s více než 120 případy (Zakharov et al., 2014). Tyto události jsou jasným důkazem toho, že hromadné akutní otravy metanolem jsou i v současné době nebezpečím pro veřejné zdraví evropských i jiných rozvinutých zemí. Vysoká úmrtnost a prevalence dlouhodobých následků otravy metanolem, zejména těžkého poškození zraku, jsou výzvou pro zdravotnické systémy z hlediska včasné diagnostiky, prognózy a prevence postižení zrakového nervu a oční sítnice u osob, které otravu přežijí.

Přestože se hromadné otravy metanolem ve světě objevují poměrně často, studie, které by poskytly kvalitní a kompletní klinická a laboratorní data

hospitalizovaných pacientů, jsou vzácné. Dosud nebyly publikovány prospektivní longitudinální kohortové studie zdravotních následků otrav u přeživších osob zaměřené na zjištění prevalence a charakteru dlouhodobého poškození zrakových funkcí, na dynamiku jejich změn v letech následujících po otravě, na asociaci s klíčovými klinickými, biochemickými a toxikologickými parametry.

2. LITERÁRNÍ PŘEHLED

2.1. Mechanismus toxického účinku metanolu na zrakový nerv a neurony oční sítnice

Metanol patří mezi látky s vysokou toxicitou intermediárních metabolitů. U člověka a primátů je metanol oxidován zejména v játrech enzymem alkoholdehydrogenázou (ADH) na formaldehyd a dále aldehyddehydrogenázou (ALDH) na kyselinu mravenčí (Eells et al., 1981a,b; Jacobsen et al, 1982; McMartin et al., 1975, 1977). Formaldehyd se v krvi neakumuluje, protože jeho konverze na kyselinu mravenčí je velmi rychlá, s poločasem 1-2 minuty (McMartin et al., 1979, 1980). Normální koncentrace kyseliny mravenčí v krevním séru nepřesahuje 0.02–0.25 mmol/L (Bouhifd et al., 2014; Osterloh et al., 1986, 1996; Psychogios et al., 2011).

Za přítomnosti koenzymu tetrahydrofolátu (THF) je kyselina mravenčí dále oxidována na kysličník uhličitý a vodu (Hantson et al., 2005; Moore et al., 1994; Shahangian et al., 1984; Tephly, 1991). Rychlost oxidace kyseliny mravenčí závisí na zásobě THF v játrech, která je u člověka (na rozdíl např. od potkanů a myší – nižších savců) relativně nízká, a na aktivitě enzymu 10-formyl-THF-dehydrogenázy, která je také přibližně dvakrát nižší než u potkanů (Aziz et al., 2002; Black et al., 1985; Eells et al., 1981b, 1982, 1983; Johlin et al., 1987; Lee et al., 1994; Noker et al., 1980). Již přibližně 15 gramů požitého metanolu plně saturuje systém oxidace kyseliny mravenčí, což vede k její akumulaci v organismu (Johlin et al., 1987; Martinasevic et al., 1996).

Kyselina mravenčí inhibuje cytochrom c oxidázu v mitochondriích (K_i~6 mmol/L) a tím vyvolává buněčnou hypoxii doprovázenou nahromaděním laktátu a poklesem ATP v buňkách (Cook et al., 2001; Erecinska & Wilson, 1980; Seme et al., 2001; Timbrell, 2000; Tong, 1982). Kumulace kyseliny mravenčí a později i kyseliny mléčné vede k rozvoji těžké metabolické acidózy (Aabakken et al., 1994; Smith et al., 1981, 2001). Existuje přímá korelace mezi koncentrací kyseliny mravenčí v séru a úmrtností na otravu metanolem (Brent et al., 2001).

Nejcitlivější k cytotoxickému účinku mravenčanu jsou neurony oční sítnice, axony zrakového nervu a neurony bazálních ganglií mozku (Sivilotti et al, 2001; Sharpe et al., 1982). Eells (Eells et al., 1996, 2000) zjistil, že koncentrace kyseliny mravenčí v očních tkaních potkanů, zejména v sítnici a ve sklivci, byla o 50 % vyšší než v mozku z důvodu pomalejší oxidace. Rozvoj těžké metabolické acidózy potencuje účinek kyseliny mravenčí na centrální nervový systém (CNS), protože usnadňuje její přechod hematoencefalickou bariérou, což vede k edému mozku a poškození (nekrózy, krvácení) v oblasti putamen, nucleus pallidus a subkortikální bílé hmoty (Blanco et al., 2006; Feany et al., 2001; Gaul et al., 1995; Zakharov et al., 2015).

Ačkoli je mechanismus toxického účinku kyseliny mravenčí v lidském těle dobře známý, údajů o prognostickém významu koncentrace kyseliny mravenčí v séru pacientů pro následky otravy metanolem je velmi málo (Sejersted et al., 1983; Tanaka et al., 1991). Koncentrace kyseliny mravenčí v séru vyšší než 500 mg/L (11 mmol/L) je často asociována s vyšším rizikem úmrtí (Ferrari et al., 2003; Jones et al., 2007; Wallage & Watterson, 2008). Nicméně Hantson dospěl k závěru, že existuje vysoká variabilita koncentrace kyseliny mravenčí u osob zemřelých na akutní otravu metanolem (Hantson et al., 2000). Hovda (Hovda et al., 2005b) uvádí, že u asymptomatických pacientů je koncentrace mravenčanu v séru v rozmezí 20-380 mg/L (0.5-8.3 mmol/L) a u pacientů s klinickými příznaky otravy je koncentrace mravenčanu běžně nad 460 mg/L (10 mmol/L). Ve studii Zakharova et al. (2015) pacienti, kteří přežili otravu metanolem a utrpěli dlouhodobé poškození zraku, měli při příjmu do nemocnice koncentraci mravenčanu v séru 16.1 (IQR 14.3–19.9) mmol/L, nebo 740 (IQR 660–920) mg/L.

2.2. Klinický obraz a léčba akutní otravy metanolem

Nejednoznačné klinické projevy metanolové otravy bývají v mnoha případech příčinou diagnostických obtíží (Hovda et al., 2005a; Paasma et al.,

2007; Zakharov et al., 2014). Po mírné ebrietě a latenci, která může trvat i 24 a více hodin (obojí závisí také na přítomnosti a množství etylalkoholu v nápoji), se objevují nejprve gastrointestinální symptomy (nauzea, zvracení, bolesti břicha), pak dušnost jako kompenzační mechanismus progredující metabolické acidózy (Bennett et al., 1953; Clay et al., 1975; Røe, 1946; Shadnia et al., 2013; Sharma et al., 2012). Pacienti si často příliš pozdě uvědomují, že jejich stav není běžnou „kocovinou“ (Naraqi et al., 1979; Paasma et al., 2007; Zakharov et al., 2014, 2015).

Poruchy vízu se projevují mlhavým viděním, poruchou barevného vidění, popřípadě světelnými záblesky a pocity oslnění, nebo naopak „zhasínáním“ a skotomy (Hovda et al., 2005a, 2005b; Coulter et al., 2011a, 2011b; Kraut, 2015). Na očním pozadí bývá přítomna hyperémie a edém v oblasti nervus opticus (Desai et al., 2013; Hassanian-Moghaddam et al., 2007; Sanaei-Zadeh, 2012). Neurologické symptomy, zejména sopor a kóma, jsou projevem těžké otravy s rozvojem edému mozku a patří mezi prognosticky nepříznivé klinické příznaky (Hovda et al., 2005a; Paasma et al., 2009; Sanaei-Zadeh, 2013; Vaneckova et al., 2014, 2015; Zakharov et al., 2016).

V léčbě akutní otravy metanolem mají klíčovou roli včasná inhibice ADH antidotem (etanolem nebo fomepizolem), korekce těžké acidemie roztokem bikarbonátu, substituce folátů (kyselina folinová nebo listová) a kontinuální nebo intermitentní hemodialýza za účelem rychlé eliminace metanolu i kyseliny mravenčí a korekce acidemie (Abramson et al., 2000; Barceloux et al., 2002; Bayliss, 2010; Beatty et al., 2013; Bekka et al., 2001; Bergeron et al., 1982; Brent, 2009, 2010; Burns et al., 1997; Chow et al., 1997; Green, 2007; Hantson P et al., 2002; Jacobsen D and McMartin KE, 1986, 1997; Kraut et al., 2008; Mycyk & Leikin, 2003; Zakharov et al., 2014, 2016, 2017).

2.3. Zrakové následky akutní otravy metanolem

Retinální gangliové buňky a jejich axony, které tvoří zrakový nerv, jsou velmi citlivé k histotoxické hypoxii způsobené inhibicí mitochondriální cytochrom c oxidázy, protože mají vyšší energetické nároky a relativně nižší počet mitochondrií (Carelli et al., 2004; Nicholls, 1976; Sharpe et al., 1982). Nicméně, biochemické a morfologické změny způsobené toxickým účinkem kyseliny mravenčí jsou přítomny i v jiných typech buněk: ve fotoreceptorech, v Müllerových buňkách aj. (Garner et al., 1995; Seme et al., 1999; Treichel et al., 2003).

Příznaky postižení zraku u pacientů s akutní otravou metanolem se projevují s určitou latencí 8-48 hodin. Doba latence závisí na množství požitého metanolu, poměru etanolu a metanolu v toxickém nápoji a jiných faktorech (Barceloux et al., 2002; Galvez-Ruiz et al., 2015a). V řadě případů dochází v průběhu několika týdnů k plnému uzdravení, k normalizaci nálezu na očním pozadí i k normalizaci zrakových funkcí (Desai et al., 2013; Sanaei-Zadeh et al., 2011; Sharma et al., 2012). Avšak dlouhodobé poškození zraku může přetrvávat u 10-30 % přeživších osob (Galvez-Ruiz et al., 2015a, 2015b; Hovda et al., 2005a; Naraqi et al., 1979; Paasma et al., 2009; Sanaei-Zadeh, 2012; Swartz et al., 1981). Dlouhodobé zrakové následky otravy zahrnují zúžení zorného pole, skotomy, snížení ostrosti zraku a kontrastní citlivosti, poruchy barvocitu až kompletní slepotu.

V longitudinální studii zrakových následků akutní otravy metanolem u 16 pacientů sledovaných po dobu 3 až 6 měsíců, Røe pozoroval další progresi poklesu ostrosti zraku u všech pacientů přeživších otravu s reziduálním zhoršením zrakových funkcí diagnostikovaným při propuštění z nemocnice (Røe, 1946). Naopak Naraqi nenašel v průběhu tří měsíců po propuštění u osmi pacientů s bilaterálním poškozením zraku signifikantní změny zrakových funkcí (Naraqi et al. 1979). Obdobně Onder nepozoroval žádné další změny u osmi pacientů, kteří přežili otravu s těžkým postižením zrakových funkcí (Onder et al., 1998). Avšak Scrimgeour uvádí případ opožděného částečného návratu

zraku po kompletní slepotě za tři měsíce po dimisi (Scrimgeour et al., 1982). Podobně, Reisin popsal případ zlepšení ostrosti zraku v průběhu dvou měsíců po otravě (Reisin et al., 1998).

Ve studii Paasma et al. (2009) všichni čtyři z 18 pacientů propuštěných z nemocnice se zrakovými následky otravy, se kterými se podařilo navázat kontakt za šest let po dimisi, měli příznaky trvalého poškození zraku, u dalších osmi z 22 pacientů propuštěných bez následků otravy byly za šest let nově zjištěny příznaky poškození zraku.

V retrospektivní studii Galvez-Ruiz et al. (2015a, 2015b), z 50 pacientů vyšetřených po odeznění akutní optické neuropatie, byl u 22 osob přítomen cupping („víčkování“) očního nervu. Všichni pacienti ze souboru měli známky atrofie očního nervu a defekty zorného pole. Autoři studie dospěli k závěru, že cupping očního nervu po akutní otravě metanolem může mít vyšší prevalenci, než se předpokládalo. Cupping očního nervu je projevem toxického účinku kyseliny mravenčí na axony a gliální buňky v prelaminární, laminární a retrolaminární oblasti.

Ve studii Dethlefs & Naraqi (1978) byla zjištěna asociace mezi incidencí dlouhodobého poškození zraku a incidencí metabolické acidózy při příjmu intoxikovaných pacientů. V další retrospektivní studii mělo pH arteriální krve při příjmu do nemocnice největší prognostický význam pro poškození zraku přetrvávající aspoň tři měsíce po propuštění z nemocnice (Desai et al., 2013).

V retrospektivní studii Sanaei-Zadeh et al. (2011) byly pozorovány tři kategorie změn zrakových funkcí u pacientů s těžkým poškozením zraku při propuštění z nemocnice: částečné zlepšení zrakových funkcí v průběhu tří až čtyř týdnů po propuštění u pěti ze 14 sledovaných pacientů, trvalá slepota bez známek zlepšení u dalších pěti pacientů a částečné zlepšení přetrvávající 1-9 měsíců s následným zhoršením zrakových funkcí, které progredovalo do úplné slepoty u dalších čtyř pacientů. Stejné případy částečného návratu zraku s následným zhoršením za 3-6 měsíců byly popsány ve studii Zhao et al. (2015).

Na základě pozorovaných rozdílů v dynamice změn zrakových funkcí v průběhu měsíců následujících po otravě, řada autorů dospěla k závěru, že dlouhodobé zrakové následky otravy metanolem jsou těžko předvídatelné (Sanaei-Zadeh et al., 2011).

2.4. Demyelinizace zrakového nervu v důsledku akutní toxické optické neuropatie

Poškození myelinového obalu zrakového nervu bylo popsáno ve studii Sharpe (1982) u čtyř pacientů zemřelých na akutní otravu metanolem. Sharpe pozoroval demyelinizaci retrolaminární části zrakového nervu bez přerušení axonů. Při akutní toxické neuropatii vede otok myelinového obalu ke kompresi axonů a poruše konduktivity nebo úplnému bloku (Hantson et al., 1999). Akutní demyelinizace zrakového nervu způsobená přímým toxickým účinkem kyseliny mravenčí může vést k axonální degeneraci v důsledku ztráty trofické podpory ze strany myelinu a přerušení normální interakce axonů s myelinem (Hantson et al., 1999; Klistorner et al., 2008; Sharpe et al., 1982).

Měření zrakových evokovaných potenciálů (VEP) může poskytnout informaci o dynamice změn konduktivity v důsledku možné regenerace, tedy remyelinizace a obnovení integrity zrakových drah (Klistorner et al., 2010; Martin M et al., 2006). Prodloužená latence zrakového evokovaného komplexu odráží stupeň demyelinizace jednotlivých axonů nervus opticus (rozsah demyelinizovaných oblastí) a následující zkrácení latence poskytuje informace o procesu remyelinizace (Jones and Brusa, 2003).

V elektrofyziologické studii akutní neuritis nervus opticus u pacientů s roztroušenou sklerózou Brusa pozoroval spontánní remyelinizaci během 2-3 let po odeznění akutní epizody (Brusa et al., 1999). Remyelinizace axonů zrakového nervu po akutním poškození myelinu může být důležitým faktorem prevence chronické ztráty axonů, retrográdní degenerace gangliových buněk sítnice a další progrese zhoršení zrakových funkcí v měsících a letech

následujících po akutní otravě. Zatím nebyly publikovány studie poskytující data o chronické remyelinizaci zrakového nervu po odeznění akutní toxické neuropatie způsobené otravou metanolem. Nicméně, možnost dlouhodobé remyelinizace axonů lze předpokládat ze studií akutní neuritis zrakového nervu u pacientů s roztroušenou sklerózou (Brusa et al., 2001; Klistorner et al., 2007, 2008, 2010).

2.5. Axonální degenerace zrakového nervu a morfologické změny vrstvy nervových vláken sítnice

Akutní toxická neuropatie nervus opticus může vést k axonální degeneraci jak v důsledku přímého účinku kyseliny mravenčí, tak i nepřímo, v důsledku myelinoklastického efektu (Barceloux et al., 2002; Sharpe et al, 1982). Tenčí axony z centrální části zorného pole jsou zranitelnější, než periferní axony většího průměru (Evangelou et al., 2001). Akutní poškození axonů může vést k retrográdní degeneraci nervových vláken a ganglií sítnice, která se projevuje ztenčením vrstvy nervových vláken (retinal nerve fiber layer, RNFL) sítnice na optické koherenční tomografii (Kornek et al., 2000).

Ve studiích akutní neuritis nervus opticus u pacientů s roztroušenou sklerózou, neuromyelitis optica a idiopatickou optickou neuritis bylo zjištěno, že axonální degenerace a pokles RNFL mohou progredovat někdy 6-12 měsíců po odeznění akutních symptomů (Costello et al., 2008, 2011; Yau et al., 2013). Tento proces je doprovázen funkčními změnami, perzistujícím poklesem amplitudy evokovaného zrakového komplexu (Klistorner et al., 2007).

Dynamika morfologických změn RNFL a změn amplitudy VEP v měsících a letech následujících po akutní toxické neuritis způsobené kyselinou mravenčí není známa. U pacientů s roztroušenou sklerózou byl po odeznění akutních příznaků neuritis pozorován nárůst amplitudy VEP v následujících 6-12 měsících přes pokračující ztrátu RNFL vláken (Klistorner et al., 2010). Autoři vysvětlují zlepšení funkčního nálezu u pacientů s progredujícím

poklesem tloušťky RNFL mechanismy neuroplasticity a remodelace funkce v postakutní fázi neuritis nervus opticus.

V případě akutní toxické neuropatie způsobené kyselinou mravenčí však histotoxická hypoxie může indukovat spuštění řady biochemických a genetických mechanismů vedoucích po odeznění akutní otravy k neuronální apoptóze (Banasiak et al., 2000).

Konečně, další faktory, jako deficit vitamínů B12 a B1, způsobený chronickým alkoholismem a/nebo malnutricí (Fei et al., 2008; Misra et al., 2003; Yeh et al., 2013), hypofunkce štítné žlázy (Fernandez et al., 2004), nedostatečně léčený diabetes mellitus mohou negativně ovlivnit mechanismy regenerace axonů zrakového nervu a vést k progresi morfologického nálezu ztráty vrstvy nervových vláken sítnice a funkčnímu nálezu poklesu amplitudy evokovaného komplexu v období po akutní otravě (Grzybowski et al., 2015; Love, 2006; Veselinovic D et al., 2005).

2.6. Asociace mezi zrakovými následky a poškozením mozku v důsledku akutní otravy metanolem

Bilaterální nekróza bazálních ganglií, zejména putamen, a hemoragické léze subkortikální bílé hmoty jsou typickými nálezy na CT nebo MRI mozku u pacientů přeživších těžkou otravu metanolem (Anderson et al., 1997; Chen et al., 1991; Giudicissi Filho et al., 1995; Jain et al., 2013; Halavaara et al., 2002; Hantson et al., 1997; Kuteifan et al., 1998; Phang et al., 1988; Roberge et al., 1998; Rubinstein et al., 1995; Taheri et al., 2010; Thirunavukkarasu et al., 2013; Vaneckova et al., 2014, 2016). Mezi jiné, méně časté nálezy, patří nekrotické změny a hemoragické léze v globus pallidus, nukleus caudate, v thalamu, mozečku a mozkovém kmeni (Bhatia et al., 2008; Chiò et al., 2004; Ganguly et al., 1996; Karayel et al., 2010; Lee et al., 2015; Penndorf-Wehner et al., 1997; Sefidbakht et al., 2007; Server et al., 2003).

Následky otravy metanolem z hlediska postižení CNS se mohou projevovat symptomy parkinsonismu (rigidita, dystonie, bradykineze, tremor, atd.), kognitivním deficitem různého stupně, nebo zůstávat subklinickými (Anderson et al., 1987; Bezdicek et al., 2014, 2016; Davis &Adair, 1999; Finkelstein & Vardi, 2002; Gille et al., 1998; Guggenheim et al., 1971; Hageman et al., 1999; Ley & Gali, 1983; LeWitt & Martin, 1988; Oliveras Ley & Gali, 1983; Reddy et al., 2007, 2010; Santos-Garcia et al., 2015). V posledním případě je problémem pozdní stanovení diagnózy a podhodnocení prevalence poškození mozku v populaci pacientů přeživších otravu, protože MRI mozku nepatří do běžného diagnostického programu v době akutní intoxikace metanolem (Blanco et al., 2006; Gaul et al., 1995; Patankar et al., 1999; Vaneckova et al., 2016; Zakharov et al., 2016).

V současné době chybí studie zabývající se asociací mezi postižením zraku a mozku v důsledku otravy metanolem. V případě přítomnosti signifikantní asociace by včasná diagnostika poškození zraku vyžadující dostupnější a méně náročná vyšetření mohla pomoci s diagnostikou CNS následků otravy u pacientů bez klinických neurologických symptomů v době dimise a vést k cílenému vyšetření zaměřenému na zjištění těchto následků (MRI mozku, cílené neurologické vyšetření, event. SPECT mozku atd.).

2.7. Problémy spojené se studií dlouhodobých zrakových následků akutních otrav metanolem

Příznaky akutní toxické neuropatie zrakového nervu způsobené kyselinou mravenčí přetrvávají delší dobu, než je doba hospitalizace. Symptomy pseudopapillitis u pacientů přeživších otravu metanolem odeznívají až za 1–2 měsíce po propuštění z nemocnice (Dethlefs & Naraqi, 1978; Ingemansson, 1984; McKellar et al., 1997; Sharpe et al., 1982). Proto aktuální funkční stav zrakových drah může být spolehlivě zhodnocen nejdříve za dva měsíce po dimisi, kdy reziduální příznaky optické neuropatie, zejména otok myelinového

obalu a s tím spojená porucha konduktivity, úplně odezní (Klistorner et al., 2007, 2008). Morfologický nález vrstvy nervových vláken sítnice (RNFL) může být také zkreslen reziduálním otokem, zejména v okolí cév, a vést k chybnému závěru, že tloušťka RNFL je normální (Klistorner et al., 2010).

Dalším problémem studie prevalence a charakteru dlouhodobých zrakových následků otrav metanolem je skutečnost, že objektivní vyšetřovací metody jako optická koherenční tomografie, zrakové evokované potenciály, magnetická rezonance se během hospitalizace u intoxikovaných pacientů běžně neindikují. Z tohoto důvodu může být podhodnocena prevalence zrakových a CNS následků u osob, které přežily otravu (Paasma et al., 2009, 2013; Patel et al., 2002; Zakharov et al., 2014, 2016).

Data o dlouhodobých zrakových následcích akutní otravy metanolem z dosavadních longitudinálních studií byla získávána za 3–6 měsíců po propuštění, z malých souborů pacientů, vyšetřených pouze jednou v době sledování bez použití objektivních metod jako OCT RNFL, VEP, MRI a standardních protokolů zahrnujících nezbytná biochemická vyšetření (vitamíny B_1, B_{12}, TSH, glykémie, aj.), proto je interpretace výsledků obtížnější a závěry nemusejí být zcela spolehlivé (Desai et al., 2013; Galvez-Ruiz et al., 2015a, 2015b; Paasma et al., 2009; Reddy et al., 2010; Sanaei-Zadeh et al., 2011, 2012).

Z uvedeného vyplývá, že studie prevalence, charakteru, závažnosti a dynamiky dlouhodobých zrakových následků otrav metanolem, zejména chronických změn zrakového nervu (remyelinizace, chronická axonální degenerace) a nervových vláken sítnice (možná retrográdní degenerace) v letech následujících po otravě vyžaduje prospektivní design, větší soubor pacientů, detailnější klinická a laboratorní data v době hospitalizace, unifikovaný diagnostický program zahrnující objektivní a subjektivní metody a minimálně tři kola vyšetření v průběhu let následujících po otravě za účelem posouzení dynamiky zjištěných změn.

3. CÍLE STUDIE

Cílem disertační práce bylo posoudit prevalenci, závažnost, charakter a dynamiku vývoje dlouhodobých zrakových následků akutní intoxikace metanolem v letech následujících po otravě.

Na základě prospektivního sledování zdravotního stavu souboru pacientů, kteří přežili akutní intoxikaci metanolem v době hromadné metanolové otravy v České republice, se předpokládá, že bude možné odpovědět na následující otázky:

1) zda a jak souvisí výskyt dlouhodobých zrakových následků intoxikace s parametry, odrážejícími závažnost akutní otravy (stav při přijetí, klinický průběh, metabolické dysbalance, hladina metanolu), a které další faktory (například druh antidota podaného k terapii akutní intoxikace nebo typ hemodialýzy, substituce foláty) jsou pro vznik těchto následků prognosticky významné;

2) jaká je skutečná prevalence a dynamika dlouhodobých zrakových následků u pacientů přeživších akutní intoxikaci metanolem a zda se jejich výskyt, závažnost a charakter vývoje liší při dimisi a s časovým odstupem v následujících letech;

3) jestli abnormální a hraniční nález OCT RNFL sítnice v průběhu let následujících po otravě progreduje, regreduje, nebo zůstává beze změn;

4) jaká je dynamika postižení zrakového nervu v důsledku myelinoklastického účinku kyseliny mravenčí; jestli je toto postižení reverzibilní nebo progredující.

4. SOUBOR PACIENTŮ A METODIKA

V době hromadné otravy metanolem v České republice v roce 2012 a v následujících letech bylo 108 pacientů hospitalizováno s potvrzenou diagnózou akutní intoxikace metanolem; 84 pacientů z této skupiny otravu přežilo. Detailní informace o okolnostech otravy, době latence, prvních příznacích a dynamice systémové toxicity a zrakových potíží byly získávány ošetřujícími lékaři při příjmu do nemocnice formou standardního dotazníku buď bezprostředně od pacientů nebo od příbuzných. Propouštěcí zprávy všech hospitalizovaných pacientů s výsledky laboratorních toxikologických a biochemických vyšetření, neurologických a očních vyšetření, CT a/nebo MRI vyšetření mozku při příjmu, v době hospitalizace a při propouštění byly analyzovány v Toxikologickém informačním středisku Kliniky pracovního lékařství Všeobecné fakultní nemocnice a 1. lékařské fakulty Univerzity Karlovy.

Při příjmu do nemocnice, byla prováděna následující laboratorní vyšetření: měření koncentrace metanolu, etanolu, kyseliny mravenčí, laktátu, glukózy, albuminu v krevním séru, funkce ledvin a jater, krevní plyny, koagulační profil (INR, APTT, trombocyty, fibrinogen), krevní obraz. Diagnóza akutní otravy metanolem byla stanovena na základě:

i) výsledků měření sérové koncentrace metanolu nad 200 mg/L (6.2 mmol/L) a informací o konzumaci tvrdých alkoholických nápojů; nebo

ii) klinických příznaků, anamnézy, detekce metanolu v krevním séru a přítomnosti nejméně dvou z následujících kritérií: pH arteriální krve <7.3, koncentrace bikarbonátu <20 mmol/L, aniontové okno >20 mmol/L.

Hospitalizovaní pacienti byli léčení v souladu s praktickým doporučením Americké akademie klinické toxikologie (AACT) a Evropské asociace toxikologických středisek a klinických toxikologů (EAPCCT). Hemodialýza byla aplikována u pacientů s následujícími kritérii: sérová koncentrace metanolu vyšší než 500 mg/L (15.6 mmol/L), metabolická acidóza (pH arteriální krve

<7.30), nebo porucha zrakových funkcí při příjmu do nemocnice. Volba modality hemodialýzy (intermitentní nebo kontinuální) záležela na několika faktorech: hemodynamická stabilita pacienta, závažnost otravy, dostupnost dialyzačních přístrojů ve zdravotnickém zařízení aj. Pacientům se závažnou acidemií (pH arteriální krve <7.20) byl podáván 8.4% nebo 4.2% roztok bikarbonátu. Jako antidotum pro blokádu alkoholdehydrogenázy byl aplikován buď etanol nebo fomepizol (4-methylpyrazol). Pro substituci vnitřní zásoby folátu pacienti dostávali kyselinu folinovou nebo kyselinu listovou.

Všichni pacienti s potvrzenou diagnózou akutní otravy metanolem, kteří přežili otravu a byli propuštění z nemocnice, byli pozváni k účasti na prospektivní longitudinální kohortové studii dlouhodobých zrakových následků otrav metanolem. Pacienti, kteří odmítli účast na klinickém vyšetření, byli vyloučení ze studie. Z 84 pacientů přeživších akutní otravu metanolem, 55 pacientů (65%) v průměrném věku 46.7 ± 3.6 let (46 mužů a 9 žen) souhlasilo s účastí ve studii a byli vyšetřeni v letech 2013-2017. Studie byla schválena Etickou komisí Všeobecné fakultní nemocnice v Praze.

Z 55 pacientů zahrnutých do prospektivní studie, 8 pacientů zemřelo dřív, než absolvovalo třetí kolo vyšetření a 5 pacientů absolvovalo méně než tři kola z důvodu pozdějšího zapojení do studie. Tito pacienti byli vyloučeni ze studie dynamiky chronických morfologických a funkčních zrakových změn. Tedy 42 pacienti, 34 muži a 8 žen, absolvovali všechna tři kola klinického vyšetření v průběhu čtyřletého sledování jejich zdravotního stavu.

Kontrolní skupina se skládala ze 41 pacientů ve věku 44.0 ± 4.2 let stejné národnosti. Podíl pacientů s chronickým alkoholismem v obou skupinách byl stejný: 23 pacienti ve sledovaném souboru a 24 pacienti v kontrolním souboru. Kritéria pro vyloučení z kontrolní skupiny byla následující: nitrooční tlak ≥22 mm Hg nebo glaukom; přítomnost reprodukovatelného výpadku zorného pole měřeného na přístroji „Medmont automated perimeter M700“ (Medmont International Pty Ltd, Vermont, Austrálie); nespolehlivý výsledek měření

zorného pole (podíl falešně pozitivních nebo falešně negativních výsledků >15% nebo ztráta fixace >20%); charakter výpadků zorného pole typický pro oční patologii; pacienti po nitroočním chirurgickém zákroku (s výjimkou katarakty a refrakční chirurgie, pokud zákrok byl proveden před více než rokem); zraková ostrost nižší než 20/32 dle škály ETDRS; známky diabetické retinopatie, diabetického makulárního edému, nebo jiná vitreoretinální onemocnění; abnormální funkce zrakového nervu nebo RNFL; použití fotosenzibilizujících léčiv v posledních dvou týdnech.

Protokol klinického vyšetření

Pacienti ze sledovaného souboru byli vyšetření celkem 3krát za 4 roky sledování: 4.9 ± 0.6 měsíců, 25.0 ± 0.6 měsíců, a 49.9 ± 0.5 měsíců po propuštění z nemocnice ve stejném zdravotnickém zařízení dle standardního klinického protokolu. Dle stejného protokolu byli vyšetřeni i pacienti z kontrolního souboru.

Standardní protokol klinického vyšetření zahrnoval následující vyšetření:

a) kompletní oční vyšetření včetně vyšetření ostrosti zraku, biomikroskopického vyšetření se štěrbinovou lampou, měření nitroočního tlaku, vyšetření očního fundu, barvocitu, perimetru, kontrastní citlivosti;

b) optická koherenční tomografie (OCT) s měřením tloušťky vrstvy nervových vláken oční sítnice (RNFL);

c) zrakové evokované potenciály (VEP);

d) magnetické rezonanční vyšetření mozku (MRI);

e) neurologické a neuropsychologické vyšetření;

f) adiktologické vyšetření;

g) genetické vyšetření (polymorfismus genů apolipoproteinu E, aldehyddehydrogenázy, systému mikrosomální oxidace etanolu (MEOS);

h) biochemické vyšetření (elektrolyty, glukóza, glykovaný hemoglobin, albumin, prealbumin, urea, kreatinin, bilirubin, jaterní enzymy, cholesterol,

triglyceridy, thyreotropní hormon (TSH), vitamíny B_1 a B_{12}, karbohydrát deficientní transferin (CDT);

i) krevní obraz a hematokrit, ethylglukuronid v moči;

j) anamnestický dotazník, dotazník kvality života SF-36.

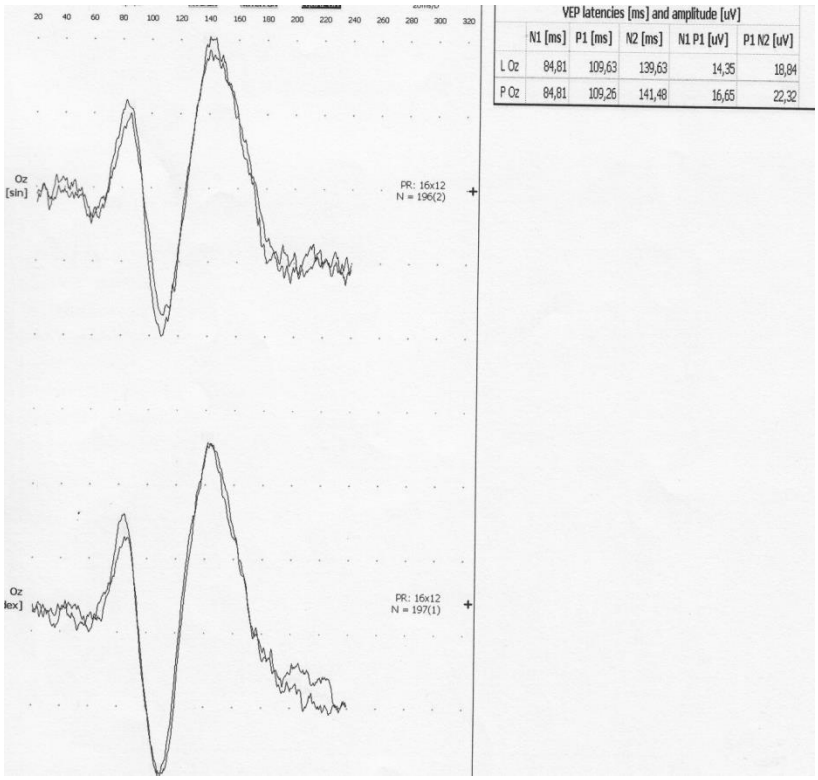
Tloušťka vrstvy nervových vláken sítnice byla měřena na přístroji „OCT Spectralis Tracking Laser Tomography“ (Heidelberg Engineering GmbH, Heidelberg, Německo, software verze 5.8.3) a porovnána s normativní databází a s výsledky měření u kontrol. Normální hodnoty byly definovány jako hodnoty spadající pod 5-95 percentil normální distribuce. Abnormální hodnoty byly definovány jako hodnoty menší než v 1. percentilu normální distribuce. Výsledky měření tloušťky RNFL globální, v nazálních a v temporálních segmentech oční sítnice byly porovnávány pro zjištění případů signifikantní ztráty tloušťky RNFL za 4 roky sledování. Ztráta globální tloušťky RNFL o 2 μm a více a/nebo ztráta aspoň 4 μm v jednom (zejména temporálním) segmentu sítnice v porovnání s výsledkem prvního měření byla považována za signifikantní.

Vyšetření VEP bylo provedeno na přístroji „TruTrace 4 Alien Technik CZ“, stimulací reverzním šachovnicovým podnětem v plném zorném poli monokulárně, se stimulační frekvencí 1.5 c/s, úhlová velikost monitoru 6^0 x 5^0 od bodu fixace, úhlová velikost čtverce 40’. Jas světlých a tmavých čtverců byl 84 cd/m^2 a 57 cd/m^2. Pásmová propust zesilovače byla 1 Hz – 1 kHz, evokovaná odpověď byla registrována ze svodu O_z-F_z dvěma kanály. Na každém oku bylo vyšetření provedeno dvakrát pro kontrolu reprodukovatelnosti evokovaného komplexu. Hodnotili jsme latenci vlny P1 a amplitudu N1P1 (Obrázek 1). Naměřené hodnoty jsme porovnávali s kontrolní skupinou a referenčními hodnotami naší laboratoře. Byla použita čtyři kritéria abnormality: (1) absence evokovaného potenciálu, (2) latence vlny P1 > 117 ms, (3) interokulární rozdíl latence vlny P1 > 6 ms, a (4) amplituda evokovaného potenciálu < 3 μV.

Výsledky byly kategorizovány jako abnormální, pokud odpovídaly aspoň jednomu ze čtyř kritérií.

Zraková ostrost byla vyšetřována pomoci standardních Snellenových tabulí z dálky 6 metrů. Za abnormální byl považován výsledek horší než 6/6. Zorné pole bylo vyšetřováno pomoci statického perimetru na přístroji „Medmont M700 automated perimeter“ (Medmont International Pty Ltd, Vermont, Austrálie). Za abnormální výsledek bylo považováno zjištění jakéhokoli defektu zorného pole. Barvocit byl vyšetřován pomoci Lanthonyho 15-D testu (Richmond Products Albuquerque NM, USA). Maximálně tři chyby byly považovány za normální nález, 3-7 chyb za hraniční a více než 7 chyb za patologický nález. Kontrastní citlivost byla vyšetřována pomoci Pelli-Robsonova testu (Clement Clarke International Ltd, Essex, U. K.). Nález byl kvalifikován jako normální (1.35 a více), hraniční (1.20-1.05), nebo patologický (méně než 1.05). Fundus byl vyšetřován pomocí biomikroskopu se štěrbinovou lampou s čočkou +78 dioptrií. Nález byl považován za patologický, pokud byly nalezeny jakékoli patologické změny disku zrakového nervu a/nebo sítnice.

Všichni pacienti se podrobili magnetickému rezonančnímu vyšetření mozku na přístroji „Gyroscan Phillips 1.5 T“ dle následujícího protokolu: axiální T2W obraz s tloušťkou řezu (THK) 6.0/0.6 mm a následujícími parametry: TR 4241 ms, TE 100 ms, FA 90°, v modu FLAIR (fluid attenuated inversion recovery): TR 11000 ms, TE 140 ms, TI 2800 ms, FA 90°, T1W obraz: TR 569 ms, TE 15 ms, FA 69°, T2W/FFE: TR 665 ms, TE 23 ms, FA 18°, SS-DWI: TR 2901 ms, TE 75 ms, FA 90°, T1W po aplikaci Gd-DTPA a koronární řez v T2W obraze s potlačením tuku (T2W-SPIR): TR 5506 ms, TE 100 ms, FA 90°.



VEP latencies [ms] and amplitude [uV]

	N1 [ms]	P1 [ms]	N2 [ms]	N1 P1 [uV]	P1 N2 [uV]
L Oz	84,81	109,63	139,63	14,35	18,84
P Oz	84,81	109,26	141,48	16,65	22,32

Obrázek 1. Normální VEP u pacienta po akutní intoxikaci metanolem

Do skupiny pacientů, kteří přežili intoxikaci metanolem s dlouhodobými zrakovými následky, byli zařazeni pacienti, kteří měli symptomy toxické optické neuropatie diagnostikované během hospitalizace, v průběhu léčby otravy byl u nich zjištěn patologický oční nález (pokles ostrosti zraku, výpadky zorného pole, abnormální barvocit a kontrastní citlivost) a měli perzistující patologický nález na fundu při propouštění z nemocnice. Za následky otravy z hlediska CNS byl považován MRI nález symetrických nekrotických a hemoragických ložisek v bazálních gangliích a subkortikální bílé hmotě kompatibilní s diagnózou akutní otravy metanolem.

5. VÝSLEDKY A DISKUZE

5.1. Prevalence a charakter zrakových následků akutních otrav metanolem (Publikace I)

Cílem naší studie bylo zjistit prevalenci a charakteru zrakových následků akutní toxické optické neuropatie v souboru pacientů přeživších otravu a propuštěných z nemocnice. Výsledky této studie se měly stát výchozím bodem pro sledování dynamiky zrakových následků v letech následujících po otravě. Celkem bylo vyšetřeno 50 pacientů za 4.9 ± 0.6 měsíců po propuštění z nemocnice (průměr a SD). Na základě provedeného vyšetření jsme zjistili, že 20 (40%) pacientů z 50 mělo zrakové následky akutní intoxikace metanolem (viz Tabulku 1), zejména abnormální funkci zrakového nervu a abnormální RNFL nález na oční sítnici. Většina pacientů se zrakovými následky otravy měla poruchu barvocitu, výpadky zorného pole, zúžení perimetru, sníženou kontrastní citlivost a patologické léze na fundu. Tři pacienti z 20 měli diabetes mellitus druhého typu, dva z nich úplně ztratili zrak během prvního dne hospitalizace s otravou, třetí pacient měl bilaterální centrální skotom, výpadky na periferii zorného pole, poruchu barvocitu, sníženou zrakovou ostrost, typický patologický nález na fundu, abnormální RNFL a VEP nález, plus symetrická bilaterální nekrotická ložiska v putamen na MRI. Dále, 10 pacientů z 20 mělo arteriální hypertenzi léčenou ACE inhibitory a jeden pacient měl sarkoidózu bez známek intraokulární sarkoidózy.

Pouze 12 z 20 pacientů (60%) se zrakovými následky otravy bylo propuštěno z nemocnice s diagnostikovaným patologickým očním nálezem v důsledku otravy metanolem, tedy u 40 % pacientů nebyl patologický nález včas zjištěn při dimisi. Podstatně více pacientů s dlouhodobými zrakovými následky mělo patologický nález na MRI mozku v porovnání s pacienty bez zrakových následků (70 % *versus* 27 %, $p<0.01$).

Tabulka 1. Výsledky prvního očního vyšetření pacientů přeživších otravu metanolem 4.9 ± 0.6 měsíců po propuštění z nemocnice.

Skupina	RNFL abnormální	VEP abnormální	Defekty zorného pole	Poruchy barvocitu	Kontrastní citlivost abnormální	Nález na očním fundu	Snížená ostrost zraku	Nález na MRI mozku
Skupina I (n=30)	1 (3%)	4 (13%)	8 (27%)	3 (10%)	11 (37%)	2 (7%)	3 (10%)	8 (27%)
Skupina II (n=20)	18 (90%)	16 (80%)	14 (70%)	14 (70%)	17 (85%)	12 (60%)	13 (65%)	14 (70%)
Celkem (n=50)	19 (38%)	20 (40%)	22 (44%)	17 (34%)	28 (56%)	14 (28%)	16 (32%)	22 (44%)
$P_{I=II}$	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	0.003

Poznámky: Skupina I – bez zrakových následků otravy; Skupina II – se zrakovými následky otravy;
RNFL – tloušťka vrstvy nervových vláken sítnice; VEP – zrakový evokovaný potenciál; MRI – magnetické rezonanční vyšetření mozku.
$P_{I=II}$ – výsledek Chi^2 testu rozdílu mezi Skupinou I a Skupinou II.

Tabulka 2. Laboratorní výsledky při příjmu do nemocnice u pacientů s akutní otravou metanolem (medián a rozmezí).

Skupina	Věk (roky)	pH	pCO_2 (kPa)	HCO_3^- (mmol/L)	BD (mmol/L)	AO (mmol/L)	S-Laktát (mmol/L)	S-MetOH (mmol/L)	S-EtOH (mmol/L)	S-Formiát (mmol/L)	S-Glukóza (mmol/L)
Skupina I	46	7.3	4.2	17.8	7.5	22.3	1.9	21.8	2.2	7.0	6.3
(n=30)	23-69	7.0-7.5	1.5-6.1	2.5-23.7	0.1-27.2	11.1-41.9	0.7-17.1	2.7-93.6	0-96.8	0-31.1	4.4-11.5
Skupina II	48	7.1	3	6.4	22.8	32.6	2.0	52.6	0	15.5	6.6
(n=20)	25-73	6.7-7.4	1.7-5.2	2.7-21.8	1.6-38.1	16.6-54.8	0.9-16.3	12.5-228.1	0-8.0	8.9-22.5	4.8-19.8
Celkem	48	7.2	3.8	11.4	16.1	24.5	1.9	28.7	0.1	11.7	6.4
(n=50)	23-73	6.7-7.5	1.5-6.1	2.5-23.7	0.1-38.1	11.1-54.8	0.7-17.1	2.7-228.1	0-96.8	0-31.1	4.4-19.8
$P_{I=II}$	0.746	0.001	0.086	0.007	0.001	0.006	0.099	0.008	0.007	0.003	0.078

Poznámky: Skupina I – bez zrakových následků otravy; Skupina II – se zrakovými následky
AO – aniontové okno; BD – deficit bází; S – krevní sérum; MetOH – metanol; EtOH – etanol.
$P_{I=II}$ – výsledek Chi^2 testu rozdílu mezi Skupinou I a Skupinou II.

Tabulka 3. Klinické příznaky otravy při příjmu do nemocnice a léčba poskytnutá pacientům s akutní otravou metanolem.

Skupina	Pohlaví (M/Ž)	Alkoholis-mus	Dávka toxického alkoholu*, mL	Doba do hospitalizace *, hodin	Zrakové příznaky	Kóma (GCS<8)	Antidotum (Fom/E)	HD (IHD/ CVVHD)	Foláty (Ano/Ne)	První pomoc etanol (Ano/Ne)
Skupina I (n=30)	25/5	19 (63%)	410 (100-1000)	24 (2-96)	7 (23%)	0	5/25 (17%/83%)	13/9 (43%/30%)	23/7 (77%/23%)	17/11 (57%/37%)
Skupina II (n=20)	16/4	13 (65%)	510 (100-1500)	34 (3-96)	13 (65%)	9	5/11 (25%/55%)	9/8 (45%/40%)	14/6 (70%/30%)	2/18 (10%/90%)
Celkem (n=50)	41/9	32 (64%)	300 (100-1500)	24 (2-96)	20 (40%)	9	10/36 (20%/72%)	22/17 (44%/34%)	37/13 (74%/26%)	19/29 (38%/58%)
$P_{I=II}$	0.963	0.419	0.420	0.685	0.012	<0.001	0.073	0.672	0.838	<0.001

Poznámky: Skupina I – bez zrakových následků otravy; Skupina II – se zrakovými následky
M – muži; Ž – ženy; GCS – Glasgow coma scale; Fom – fomepizol; E – etanol; HD – hemodialýza; IHD – intermitentní hemodialýza; CVVHD – kontinuální hemodialýza. $P_{I=II}$ – výsledek Chi^2 testu rozdílu mezi Skupinou I a Skupinou II.
* - medián a rozmezí.

Tabulka 4. Univariántní a multivariántní logistická regrese laboratorních parametrů při příjmu do nemocnice signifikantních pro poškození axonů zrakového nervu u pacientů s akutní otravou metanolem.

	Před adjustací		Po adjustaci	
Parametr	OR	(95% CI)	OR	(95% CI)
S-EtOH	**0.10**	**(0.02 - 0.52)**	**0.07**	**(0.01 - 0.8)**
S-MetOH	**4.25**	**(1.52 - 11.94)**	**5.59**	**(1.49 - 21.03)**
pH	**2.41**	**(1.35 - 4.32)**	**3.92**	**(1.59 - 25.95)**
HCO_3^-	**2.35**	**(1.14 - 4.84)**	1.16	(0.01 - 2.49)
S-Laktát	1.82	(0.69 - 4.8)	1.43	(0.36 - 5.65)

Poznámky: Tučně zvýrazněná čísla indikují statisticky významný výsledek ($p < 0.05$). OR - odds ratio; CI – konfidenční interval; S- sérum; EtOH – etanol (nedetekovatelný *versus* pozitivní koncentrace); MetOH – metanol (<15 mmol/L, 15-25 mmol/L, >25 mmol/L); pH – arteriální pH při příjmu (<7.14, 7.14-7.25, >7.25); HCO_{3-} - bikarbonát (<9.3 mmol/L, 9.3-13.8 mmol/L, >13.8 mmol/L); Laktát – koncentrace laktátu v krevním séru (<1.2 mmol/L, 1.2-2.9 mmol/L, >2.9 mmol/L).

Symetrická nekrotická ložiska v putamen byla přítomna u 58 % pacientů se zrakovými následky a pouze u 10 % pacientů bez postižení zraku ($p < 0.01$).

Výsledky laboratorních vyšetření a klinické příznaky při příjmu do nemocnice jsou uvedeny v tabulkách 2 a 3. Pět laboratorních parametrů, pH arteriální krve, koncentrace bikarbonátu, laktátu, metanolu a etanolu při příjmu byly použity pro univariántní a multivariántní analýzu za účelem predikce pravděpodobnosti poškození axonů zrakového nervu u pacientů s toxickou optickou neuropatií (Tabulka 4). Nebyla nalezena asociace mezi druhem antidota aplikovaného v nemocnici (etanol nebo fomepizol), substitucí folátu, a postižením zrakového nervu v důsledku otravy.

V rámci studie jsme zjistili, že prevalence dlouhodobých zrakových následků akutních otrav metanolem je podstatně vyšší, než byla stanovena při propuštění z nemocnic a než byla doposud popsána v literatuře. Z 37 pacientů bez

subjektivních zrakových potíží byl u 8 (22%) pacientů zjištěn abnormální RNFL nález oční sítnice a abnormální VEP nález.

Otravy metanolem jsou doprovázeny vysokou mortalitou a výskytem závažných zdravotních následků přes nákladnou a složitou léčbu (Barceloux et al., 2002). Postižení zraku u pacientů přeživších otravu může být dočasným nebo trvalým s různou dynamikou (Desai et al., 2013; Galvez-Ruiz et al., 2015a,b; Paasma et al., 2009; Sanaei-Zadeh et al., 2011; Scrimgeour et al., 1982). Vyšší prevalence zrakových následků v naší studii v porovnání s výsledky jiných studií (Hovda et al., 2005a; Naraqi et al., 1979; Swartz et al., 1981), stejně jako značný výskyt subklinických abnormálních nálezů nedoprovázených subjektivními příznaky, svědčí o nezbytnosti komplexního očního vyšetření u každého pacienta přeživšího otravu metanolem při propuštění z nemocnice. Toto vyšetření musí zahrnovat nejen standardní testy, ale také vyšetření OCT RNFL a VEP.

Závažnost metabolické acidózy, koncentrace metanolu a etanolu v krevním séru při příjmu do nemocnice demonstrovaly signifikantní asociaci se zrakovými následky otravy metanolem. Dřívější studie uváděly jen roli pH jako klíčového prognostického faktoru pro poškození zraku (Desai et al., 2013; Sanaei-Zadeh et al., 2011). Pacienti s poškozením zraku měli dvakrát vyšší koncentraci mravenčanu v krvi při příjmu do nemocnice v porovnání s pacienty bez poškození zrakových funkcí. Dlouhodobé zrakové následky byly zjištěny u všech 9 pacientů přijatých do nemocnice v kómatu (GCS < 8), z nichž 4 (44 %) úplně ztratili zrak. Kóma při příjmu je negativní prognostický znak pro přežití otravy metanolem (Liu et al., 1998; Paasma et al., 2012).

Analýza klinických příznaků ukazuje, že pacienti se symptomy poruchy zraku při příjmu do nemocnice měli zrakové následky otravy mnohem častěji než pacienti bez subjektivních potíží při příjmu (65 % *versus* 23 %, $p<0.05$). Ve studii

Sanaei-Zadeh H et al. (2011) rané subjektivní symptomy zrakové toxicity jako rozmazané vidění ustupovaly do dvou týdnů. Akutní pseudopapillitis s typickým edémem disku očního nervu a edémem peripapilární oční sítnice úplně ustupuje v průběhu několika týdnů (Galvez-Ruiz et al., 2015a, 2015b; Ingemansson, 1984; Dethlefs and Naraqi, 1978).

Na druhé straně, zpomalení konduktivity očního nervu, pokles amplitudy VEP a abnormální tloušťka RNFL představují dlouhodobé důsledky demyelinizace očního nervu a axonální degenerace oční sítnice (Brusa et al., 2001; Klistorner et al., 2008). Dynamika těchto procesů v letech následujících po otravě a jejich klinické determinanty byly předmětem naší prospektivní studie, jejíž výsledky jsou popsány v následujících kapitolách.

5.2. Progredující chronická degenerace axonů zrakového nervu a nervových vláken oční sítnice: morfologické a elektrofyziologické parametry, vliv na zrakové funkce (Publikace II, III)

Znalosti charakteru postižení a determinant ovlivňujících chronické morfologické změny oční sítnice jsou klíčové pro hodnocení účinnosti léčby, prognózu, posouzení potřeby speciálních zdravotních přístrojích a zlepšení kvality života pacientů přeživších otravu metanolem. Proto jsme uskutečnili prospektivní longitudinální kohortovou studii se sérií měření tloušťky vrstvy nervových vláken sítnice u 42 pacientů v průběhu čtyř let po akutní intoxikaci metanolem a u 41 kontrol. Demografické a laboratorní ukazatelé pacientů z obou souborů jsou uvedeny v tabulce 5.

Při příjmu do nemocnice měli pacienti ze sledovaného souboru koncentraci metanolu v séru 1.43±0.47 g/L, kyseliny mravenčí 0.60±0.15 g/L, pH arteriální krve 7.17±0.07, a deficit bází 16.50±3.50 mmol/L (průměr a SD). Polovina pacientů ze sledovaného souboru měla při příjmu závažnou metabolickou acidózu, 10 pacientů bylo přijato v kómatu, 20 pacientů mělo zrakové potíže v rozmezí od rozmazaného vidění do kompletní slepoty (3 pacienty).

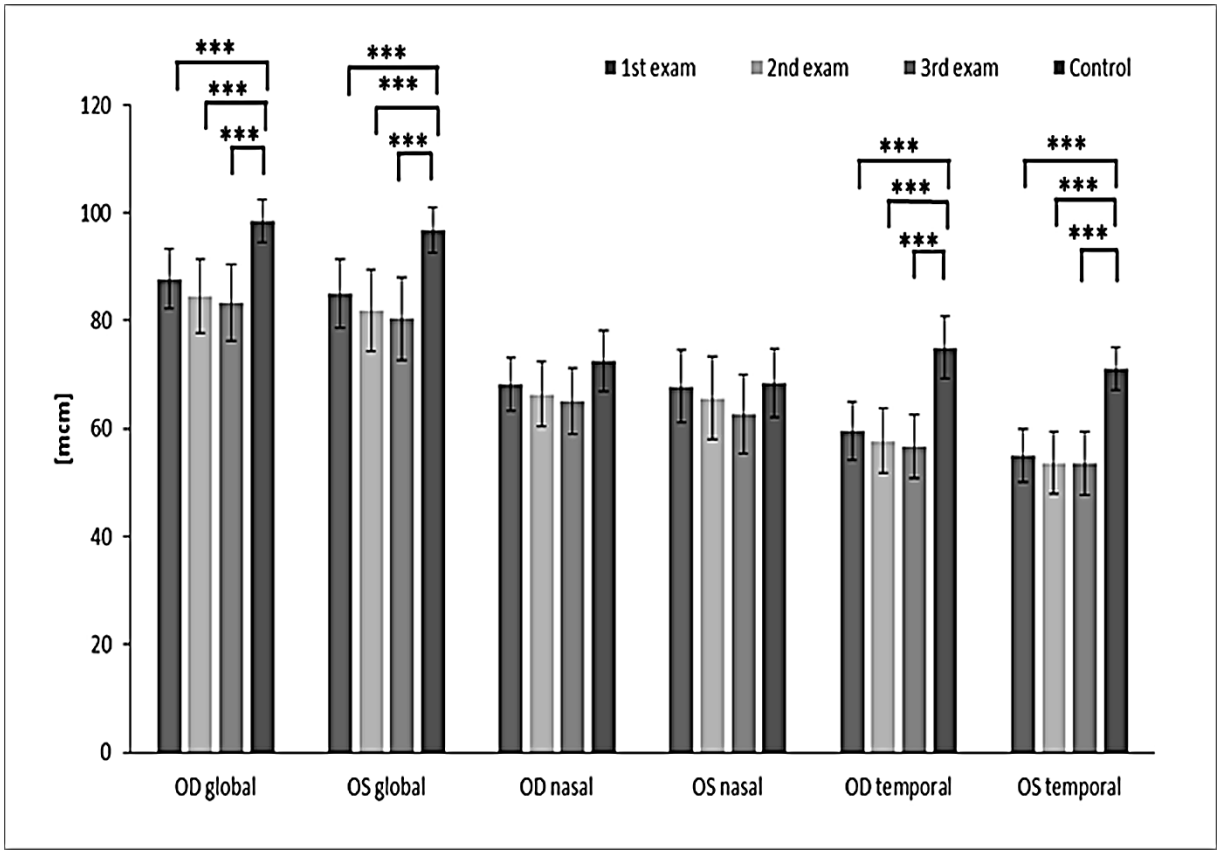
Dynamika chronického poklesu tloušťky vrstvy nervových vláken sítnice

Výsledky třech měření tloušťky RNFL v průběhu 4 let jsou uvedeny na obrázku 2. Signifikantní pokles globální tloušťky RNFL s největší ztrátou v temporálních segmentech byl zaznamenán ve sledovaném souboru v porovnání s kontrolami.

Tabulka 5. Demografické a laboratorní ukazatele pacientů ze sledovaného a kontrolního souborů (průměr a SD) v rámci studie chronických morfologických změn oční sítnice.

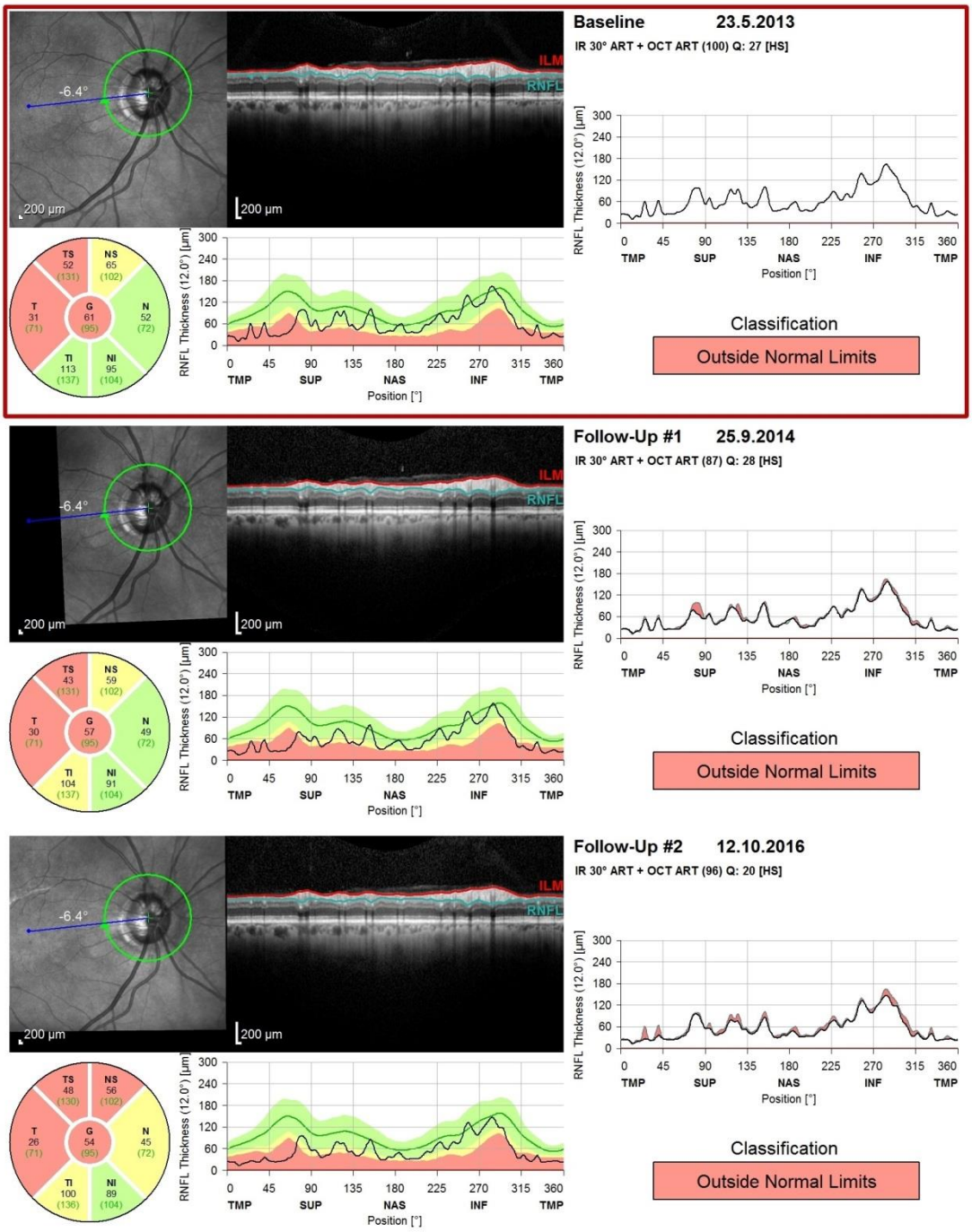
	Věk, roky	Abusus alkoholu	Glukóza, mmol/L	Clyc HbA1c, mmol/mol	Kreatinin, µmol/L	Cholesterol, mmol/L	TSH, mIU/L	Triacylgly-ceridy, mmol/L	Vitamín B_1, µg/L	Vitamín B_{12}, µg/L	GGT, µkat/L
Sledovaný soubor (n=42)	45.7±4.4	23 (55%)	5.2±0.3	33.9±2.1	79.5±4.5	5.4±0.4	2.3±0.3	1.5±0.3	60.8±5.5	394±51	1.9±0.8
Kontrolní soubor (n=41)	44.0±4.2	24 (59%)	4.6±0.3	36.0±1.5	76.0±9.4	4.6±0.4	2.1±0.6	1.6±1.0	45.7±4.2	420±110	0.6±0.3
P	**0.729**	**0.426**	0.004	**0.102**	**0.507**	0.008	**0.554**	**0.859**	0.001	**0.687**	0.006

Poznámka: rozdíl $p<0.05$ byl považován za signifikantní (zvýrazněno tučně)

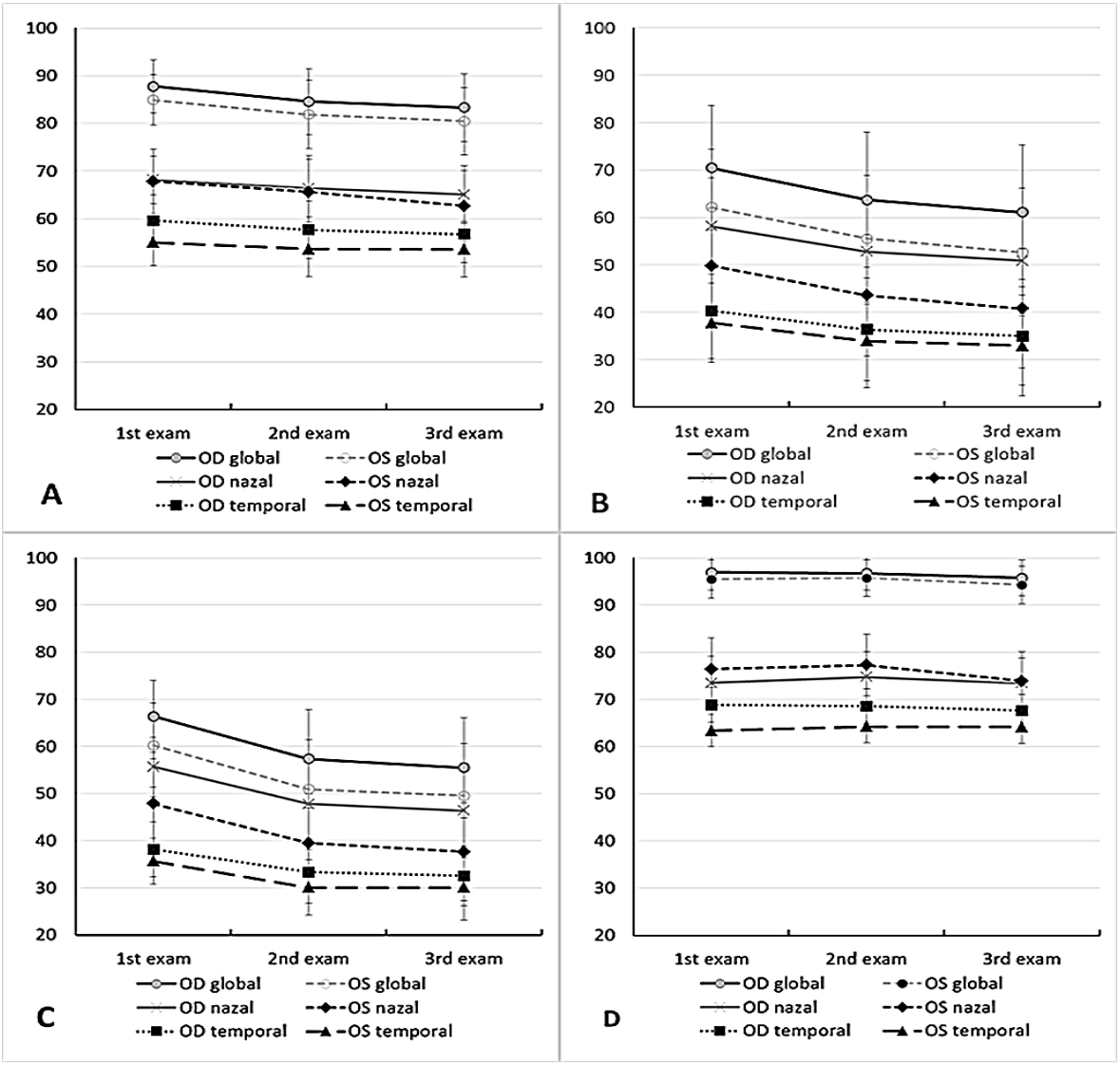


Obrázek 2. Výsledky měření RNFL ve sledovaném souboru (n=42) v průběhu 4 let versus výsledky měření u kontrol (n=41).
*Poznámky: OD – oculus dexter; OS – oculus sinister; *** - p<0.001; exam – jednotlivá měření 4.9[±0.6] měsíců, 25.0[±0.6] měsíců, a 49.9[±0.5] měsíců po propouštění z nemocnice (průměr a SD).*

Příklad individuální dynamiky RNFL v průběhu studie je uveden na obrázku 3. Rychlost poklesu RNFL byla signifikantně vyšší než rychlost fyziologického poklesu spojeného se stárnutím (obrázek 4 nahoře vlevo). Nejvyšší rychlost ztráty axonů byla zaznamenána u pacientů se závažnou intoxikací a metabolickou acidózou při příjmu (obrázek 4 nahoře vpravo). Tito pacienti měli velmi nízkou koncentraci etanolu nebo negativní etanol v séru při příjmu do nemocnice, nižší pH arteriální krve a vyšší koncentraci kreatininu v porovnání s pacienty bez signifikantního poklesu RNFL v době sledování (Tabulka 6).



Obrázek 3. Individuální dynamika poklesu tloušťky RNFL u pacienta v době sledování.



Obrázek 4. Dynamika poklesu tloušťky RNFL v průběhu 4 let po akutní otravě metanolem ve sledovaném souboru (nahoře vlevo), u pacientů se závažnou otravou (nahoře vpravo), s abnormálním (dole vlevo) a normálním (dole vpravo) RNFL nálezem při prvním měření.
*Poznámky: OD – oculus dexter; OS – oculus sinister; *** - p<0.001; exam – jednotlivá měření 4.9[±0.6] měsíců, 25.0[±0.6] měsíců, a 49.9[±0.5] měsíců po propouštění z nemocnice (průměr a SD).*

Pacienti s abnormálním RNFL nálezem při prvním měření měli nejvyšší rychlost ztráty axonů v období mezi prvním a druhým měřením (obrázek 4 dole vlevo). Konečně, pacienti s normálním nebo hraničním nálezem RNFL při prvním

Tabulka 6. Demografické, toxikologické a biochemické parametry u pacientů se signifikantním chronickým poklesem tloušťky RNFL ve sledovaném období *versus* pacienti bez signifikantního poklesu RNFL (průměr a SD).

	Věk, roky	MetOH, mg/L	EtOH, mg/L	pH	Kreatinin, µmol/L	Glukóza, mmol/L	Laktát, mmol/L	Mravenčan, mg/L	Vitamín B_1, µg/L	Vitamín B_{12}, µg/L	GGT, µkat/L
Pokles RNFL (n=10)	50.0±9.2	1950±980	73±95	7.00±0.19	131±32	11.4±4.5	6.0±3.4	620±460	68±18	480±170	0.9±0.6
Bez poklesu RNFL (n=32)	44.4±5.3	1250±550	280±180	7.22±0.07	83.3±9.1	7.2±1.0	2.4±1.0	600±170	57.8±5.4	413±65	1.1±0.4
P	0.290	0.201	**0.042**	**0.005**	**0.010**	0.078	0.053	0.859	0.272	0.384	0.444

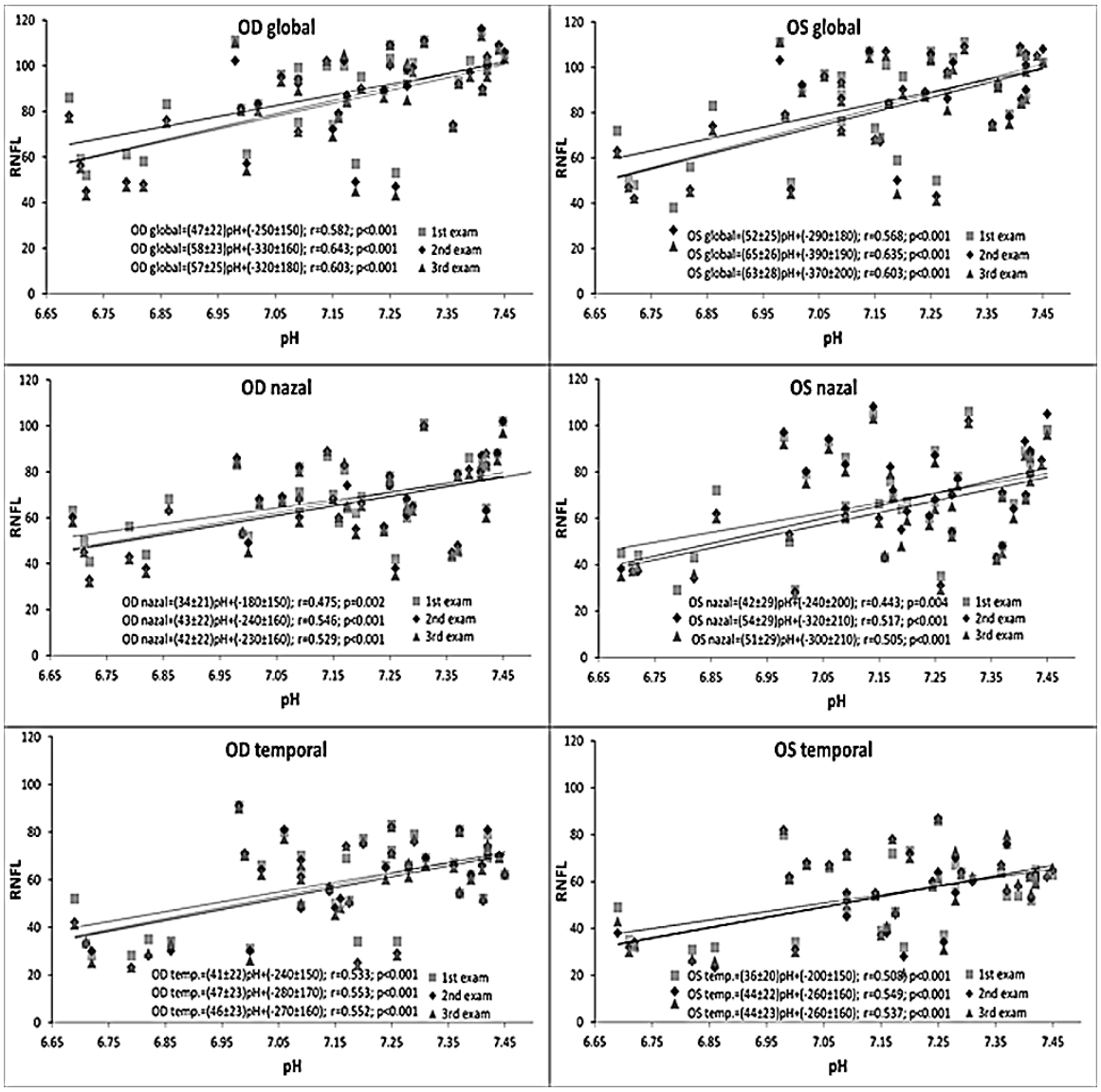
Poznámka: rozdíl $p<0.05$ byl považován za signifikantní (zvýrazněno tučně)

měření neměli negativní dynamiku změn tloušťky RNFL v průběhu 4 let sledování (obrázek 4 dole vpravo). Interokulární rozdíly v průměrné tloušťce RNFL ve sledovaném souboru byly signifikantní pro globální a temporální RNFL ve všech třech kolech měření, což bylo vzato v úvahu v multivariántním modelu jako proměnná „OD/OS rozdíl v RNFL tloušťce“.

Ukazatel závažnosti metabolické acidózy, pH arteriální krve při příjmu do nemocnice, demonstroval nejsignifikantnější asociaci jak s tloušťkou RNFL (obrázek 5), tak i s dynamikou chronické ztráty axonů (obrázek 6). V bivariántních modelech byla nalezena signifikantní asociace tloušťky RNFL s pohlavím, věkem, sérovou koncentrací metanolu, etanolu, mravenčanu, laktátu, glukózy při příjmu do nemocnice a s koncentrací vitamínů B_1 a B_{12} měřenou ve sledovaném období (všechna $p < 0.05$). Nebyla nalezena asociace tloušťky RNFL s koncentrací glukózy, glykovaného hemoglobinu, cholesterolu, triacylglyceridů, TSH a dalšími laboratorními biochemickými parametry měřenými ve sledovaném období.

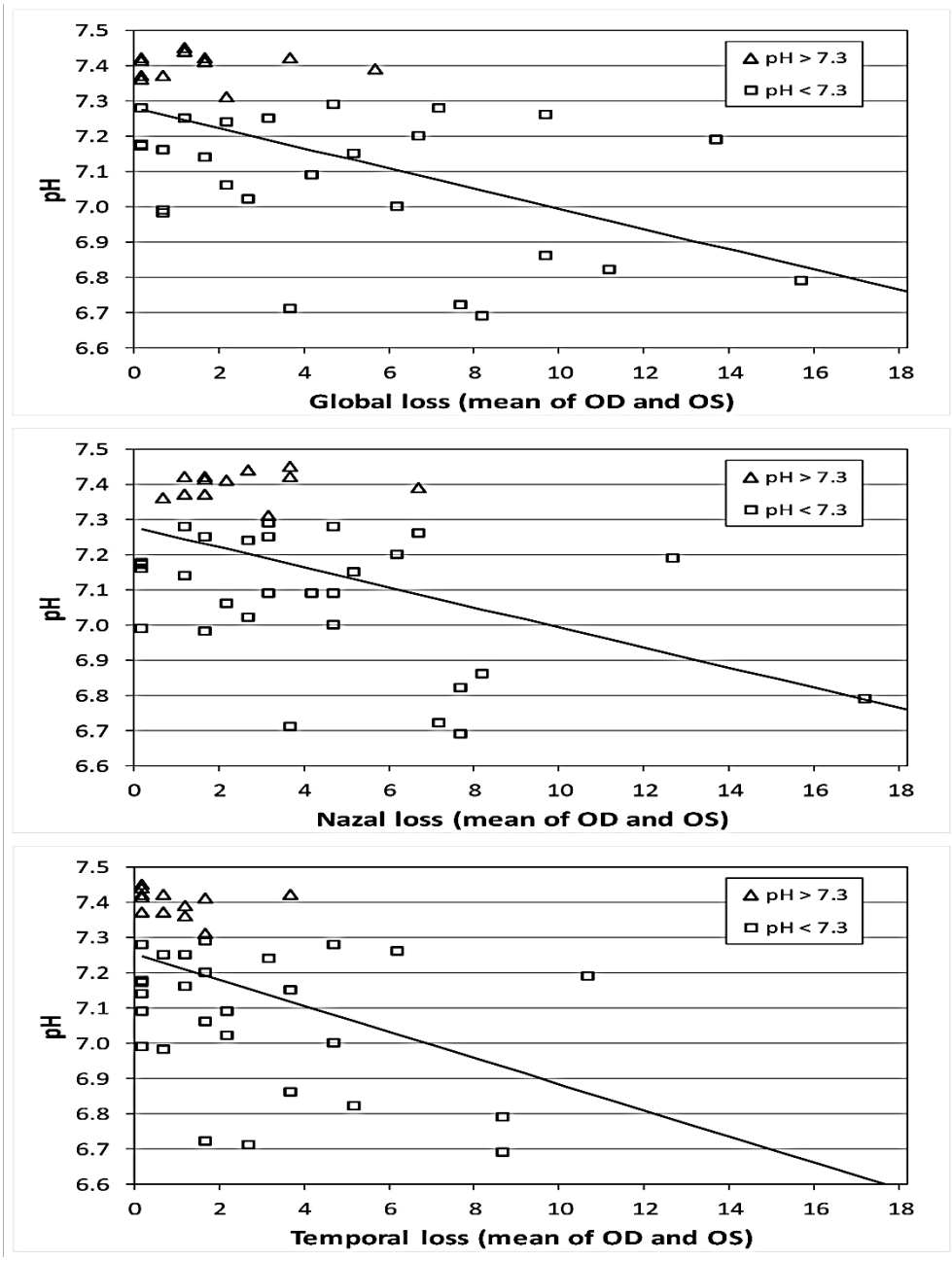
Z terapeutických opatření byla zjištěna asociace mezi přednemocničním podáním etanolu jako „první pomoci“ a větší tloušťkou globální RNFL ($r = 0.388$ a 0.355; $p = 0.010$ a 0.030 pro OD a OS), jakož i menší dynamikou chronické ztráty axonů ($r = –0.396$ a $–0.406$; $p = 0.013$ a 0.010 pro OD a OS). Pacienti, u kterých byla aplikována intermitentní hemodialýza, měli menší ztrátu axonů v porovnání s pacienty léčenými pomoci kontinuální hemodialýzy ($r = 0.473$ a 0.367; $p = 0.002$ a 0.017 pro OD a OS). Nebyla nalezena asociace mezi tloušťkou RNFL, dynamikou ztráty axonů a jinými léčebnými modalitami (aplikace etanolu nebo fomepizolu v nemocnici, substituce foláty, aj.).

Pro regresní analýzu dynamiky poklesu tloušťky globální RNFL ve sledovaném období byly následující proměnné zahrnuty do modelu jako prediktory: věk, pohlaví, doba od ingesce metanolu, OD / OS rozdíl v tloušťce RNFL, laboratorní parametry měřené při příjmu do nemocnice s akutní otravou (pH



Obrázek 5. Asociace tloušťky globální RNFL (nahoře vlevo, nahoře vpravo), RNFL v nazálním segmentu (uprostřed vlevo, uprostřed vpravo) a RNFL v temporálním segmentu (dole vlevo, dole vpravo) měřené třikrát v období sledování a pH arteriální krve při příjmu do nemocnice s akutní otravou metanolem.

Poznámka: OD – oculus dexter; OS – oculus sinister.



Obrázek 6. Asociace dynamiky chronického poklesu tloušťky globální (nahoře), nazální (uprostřed) a temporální (dole) RNFL v průběhu 4 let sledování a pH arteriální krve při příjmu do nemocnice s akutní otravou metanolem.

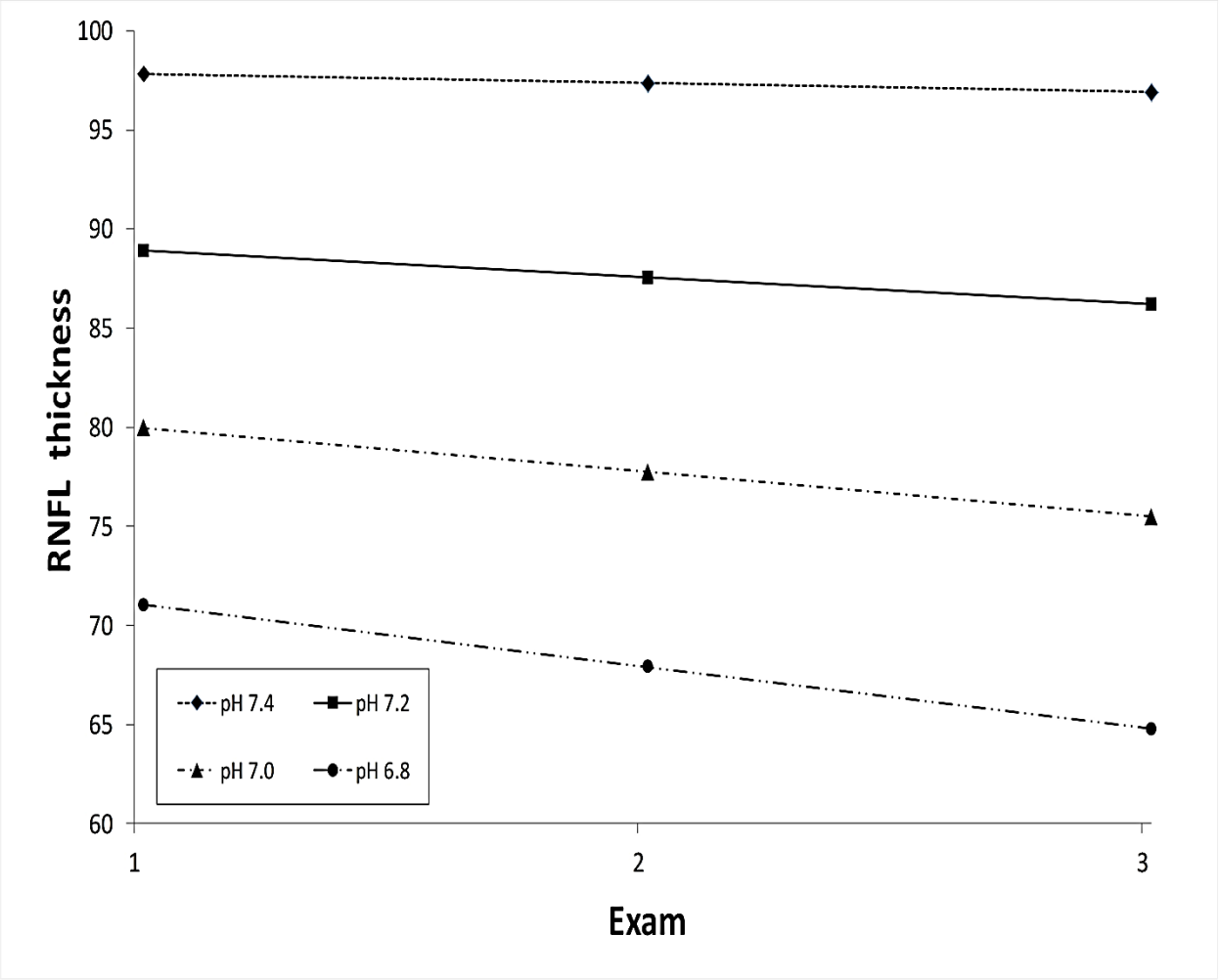
Poznámka: OD – oculus dexter; OS – oculus sinister

Tabulka 7. Model předpovídající změny tloušťky globální RNFL v průběhu 4 let sledování.

Parametr	Odhad	Standardní odchylka	df	t	Významnost	95% Konfidenční Interval	
						Dolní hranice	Horní hranice
Intercept	87.47	3.52	24.94	24.83	**0.000**	80.22	94.73
Čas	-0.75	0.12	32.04	-6.12	**0.000**	-0.99	-0.50
OD/OS rozdíl	1.35	0.37	40.64	3.63	**0.001**	0.60	2.11
Pohlaví	-0.74	3.80	24.90	-0.19	0.848	-8.57	7.09
Věk	-3.38	3.10	24.99	-1.09	0.286	-9.76	3.00
pH	9.66	3.36	25.03	2.88	**0.008**	2.74	16.57
Čas*pH	0.48	0.12	33.34	3.99	**0.000**	0.24	0.73
MetOH	-3.94	4.24	24.95	-0.93	0.361	-12.67	4.79
EtOH	1.87	2.98	25.06	0.63	0.536	-4.26	8.00
Glukóza	-0.33	0.48	99.62	-0.70	0.487	-1.28	0.61
B_1_1.	-0.29	3.30	25.02	-0.09	0.931	-7.08	6.51
B_1_2.	-5.81	2.91	25.00	-2.00	0.057	-11.81	0.18
TSH	0.02	0.16	80.13	0.10	0.917	-0.30	0.33
B_{12}_1.	-2.63	2.77	24.90	-0.95	0.351	-8.33	3.07
B_{12}_2.	-0.31	5.35	24.95	-0.06	0.954	-11.34	10.71
B_{12}_3.	0.08	5.38	24.95	0.01	0.988	-11.01	11.17

*Poznámky: Čas – délka časového intervalu od požití metanolu do začátku hospitalizace; OD/OS rozdíl – interokulární rozdíl v průměrné tloušťce globální RNFL; pH – pH arteriální krve při příjmu; Čas*pH – efekt interakce mezi pH arteriální krve a časem od ingesce metanolu do začátku hospitalizace; MetOH – koncentrace metanolu; EtOH – koncentrace etanolu; Glukóza – koncentrace glukózy; B1 – koncentrace vitamínu B_1; B_{12} – koncentrace vitamínu B_{12}; TSH – koncentrace thyreotropního hormonu; 1., 2., 3. - jednotlivá měření 4.9[±0.6] měsíců, 25.0[±0.6] měsíců, a 49.9[±0.5] měsíců po propouštění z nemocnice (průměr a SD).*

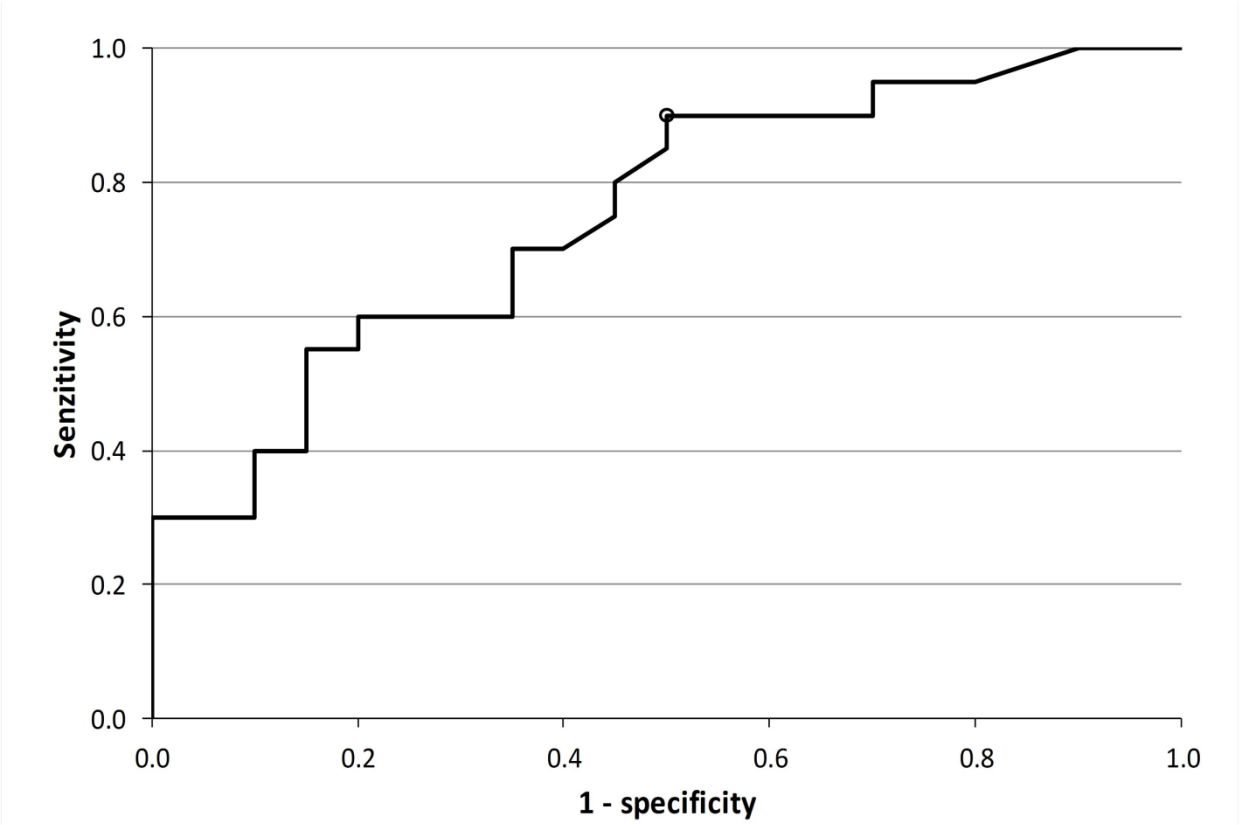
arteriální krve, koncentrace metanolu, etanolu a glukózy), koncentrace vitamínů B_1, B_{12}, TSH měřené v období sledování. Prognostický model poklesu tloušťky globální RNFL v průběhu čtyř let po akutní toxické optické neuropatie pro různé hodnoty pH arteriální krve po adjustaci na ostatní nezávislé proměnné použité pro multivariántním analýzu (Tabulka 7) je uveden na obrázku 7.



Obrázek 7. Prognostický model poklesu tloušťky globální RNFL v době sledování v závislosti na pH arteriální krve po adjustaci na další nezávislé proměnné (viz Tabulku 7).

Poznámky: RNFL thickness – průměrná predikovaná tloušťka globální RNFL, μm; pH – pH arteriální krve při příjmu; Exam - jednotlivá měření 4.9[±0.6] měsíců, 25.0[±0.6] měsíců, a 49.9[±0.5] měsíců po propouštění z nemocnice (průměr a SD).

Křivka „Receiver Operating Characteristic“ (ROC) předpovídající pokles tloušťky globální RNFL v období sledování v závislosti na pH arteriální krve při příjmu po adjustaci na věk a pohlaví je uvedena na obrázku 8. Pro tento model „Area Under the Curve“ (AUC) je 0.753 ± 0.076 ($p = 0.006$) je senzitivita 90 % a specificita je 50 %. Odhad rizika signifikantního poklesu tloušťky globální RNFL (více 2 μm) v průběhu let po akutní optické neuropatii v důsledku otravy metanolem pro pH arteriální krve pod 7.3 je: OR 9.0 (1.64–49.47 CI 95%, $p < 0.05$) a OR 11.65 (1.91–71.12 CI 95%, $p < 0.05$) po adjustaci na věk a pohlaví.



Obrázek 8. ROC křivka předpovídající pokles tloušťky globální RNFL v období sledování v závislosti na pH arteriální krve při příjmu.

Dynamika poklesu tloušťky RNFL a funkce očního nervu dle měření VEP.

Při prvním vyšetření byla abnormální latence vlny P1 zrakových evokovaných potenciálů naměřena u 18 pravých očí (43 %) a 21 levých očí (50 %), včetně 5 pravých očí a 4 levých očí s nevybavitelným evokovaným potenciálem. Korelace mezi tloušťkou globální a temporální RNFL, P1 latencí a amplitudou N1P1 evokovaných potenciálů je uvedena v tabulce 8. Negativní korelace mezi latencí P1 a tloušťkou RNFL (tedy více prodloužená latence P1 odpovídá menší tloušťce RNFL) byla nejvýznamnější při prvním vyšetření, méně významná při druhém vyšetření a konečně nevýznamná při třetím vyšetření. Postupná ztráta významnosti korelace odpovídá postupnému procesu remyelinizace axonů zrakového nervu.

Pozitivní korelace mezi tloušťkou RNFL a amplitudou N1P1 evokovaného potenciálu (tedy vyšší amplituda odpovídá větší tloušťce RNLF) demonstrovala opačnou tendenci: nejvýznamnější korelace byla při posledním, třetím vyšetření (Tabulka 8). Dynamika poklesu tloušťky globální RNFL pozitivně korelovala s latencí P1 evokovaného potenciálu měřenou během prvních dvou vyšetření: rychlost chronické ztráty axonů byla vyšší u pacientů s delší latencí P1 v důsledku akutní demyelinizace zrakového nervu (OD: $r = 0.392$ a 0.396; OS: $r = 0.322$ a 0.386 pro 1. a 2. vyšetření, všechna $p < 0.05$). Konečně, rychlost chronické ztráty axonů negativně korelovala s amplitudou N1P1 evokovaných potenciálů měřených během všech třech vyšetření. Pacienti s nižší amplitudou N1P1 evokovaného potenciálu měli vyšší rychlost ztráty axonů zrakového nervu.

Tabulka 8. Korelace tloušťky RNFL, latence P1 a amplitudy N1P1 zrakových evokovaných potenciálů.

		OD globální RNFL 1. vyšetření	OD globální RNFL 2. vyšetření	OD globální RNFL 3. vyšetření	OS globální RNFL 1. vyšetření	OS globální RNFL 2. vyšetření	OS globální RNFL 3. vyšetření	OD temporální RNFL 1. vyšetření	OD temporální RNFL 2. vyšetření	OD temporální RNFL 3. vyšetření	OS temporální RNFL 1. vyšetření	OS temporální RNFL 2. vyšetření	OS temporální RNFL 3. vyšetření
OD P1 1. vyšetření	Pearson Correlation	-0.424	-0.426	-0.420	-0.468	-0.489	-0.475	-0.540	-0.530	-0.557	-0.508	-0.502	-0.463
	Sig. (2-tailed)	**0.010**	**0.010**	**0.011**	**0.005**	**0.002**	**0.002**	**0.001**	**0.001**	**0.000**	**0.002**	**0.002**	**0.004**
OD P1 2. vyšetření	Pearson Correlation	-0.411	-0.438	-0.441	-0.461	-0.506	-0.499	-0.447	-0.469	-0.510	-0.490	-0.492	-0.471
	Sig. (2-tailed)	**0.014**	**0.009**	**0.008**	**0.005**	**0.002**	**0.002**	**0.007**	**0.004**	**0.002**	**0.003**	**0.003**	**0.004**
OD P1 3. vyšetření	Pearson Correlation	0.106	0.111	0.105	0.166	0.136	0.133	0.136	0.107	0.127	0.083	0.077	0.083
	Sig. (2-tailed)	0.531	0.513	0.537	0.327	0.421	0.432	0.421	0.530	0.452	0.625	0.649	0.627
OS P1 1. vyšetření	Pearson Correlation	-0.535	-0.528	-0.548	-0.595	-0.588	-0.604	-0.517	-0.510	-0.554	-0.497	-0.487	-0.503
	Sig. (2-tailed)	**0.001**	**0.001**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.001**	**0.000**	**0.002**	**0.002**	**0.001**
OS P1 2. vyšetření	Pearson Correlation	-0.521	-0.533	-0.549	-0.457	-0.484	-0.502	-0.463	-0.506	-0.535	-0.408	-0.430	-0.450
	Sig. (2-tailed)	**0.001**	**0.001**	**0.001**	**0.005**	**0.003**	**0.002**	**0.004**	**0.002**	**0.001**	**0.013**	**0.009**	**0.006**
OS P1 3. vyšetření	Pearson Correlation	-0.027	-0.034	-0.040	0.029	-0.009	-0.016	0.001	-0.046	-0.026	-0.046	-0.069	-0.065
	Sig. (2-tailed)	0.877	0.845	0.819	0.869	0.959	0.926	0.996	0.791	0.879	0.789	0.688	0.707
OD N1P1 1. vyšetření	Pearson Correlation	0.273	0.296	0.356	0.393	0.385	0.419	0.461	0.463	0.495	0.505	0.473	0.495
	Sig. (2-tailed)	0.107	0.080	**0.033**	**0.018**	**0.020**	**0.011**	**0.005**	**0.004**	**0.002**	**0.002**	**0.004**	**0.002**

OD N1P1 2. vyšetření	Pearson Correlation	0.250	0.256	0.321	0.414	0.402	0.434	0.381	0.389	0.438	0.448	0.456	0.468
	Sig. (2-tailed)	0.147	0.138	0.060	**0.013**	**0.017**	**0.009**	**0.024**	**0.021**	**0.008**	**0.007**	**0.006**	**0.005**
OD N1P1 3. vyšetření	Pearson Correlation	0.253	0.254	0.312	0.399	0.373	0.400	0.397	0.383	0.432	0.463	0.453	0.457
	Sig. (2-tailed)	0.130	0.130	0.060	**0.015**	**0.023**	**0.014**	**0.015**	**0.019**	**0.008**	**0.004**	**0.005**	**0.004**
OS N1P1 1. vyšetření	Pearson Correlation	0.410	0.431	0.432	0.428	0.420	0.426	0.291	0.297	0.306	0.320	0.309	0.316
	Sig. (2-tailed)	**0.011**	**0.007**	**0.007**	**0.007**	**0.009**	**0.008**	0.076	0.070	0.062	0.050	0.059	0.053
OS N1P1 2. vyšetření	Pearson Correlation	0.309	0.315	0.375	0.428	0.416	0.444	0.412	0.426	0.474	0.435	0.438	0.453
	Sig. (2-tailed)	0.066	0.061	**0.024**	**0.009**	**0.012**	**0.007**	**0.013**	**0.010**	**0.003**	**0.008**	**0.007**	**0.006**
OS N1P1 3. vyšetření	Pearson Correlation	0.304	0.314	0.370	0.451	0.432	0.458	0.394	0.397	0.450	0.452	0.451	0.470
	Sig. (2-tailed)	0.072	0.062	**0.026**	**0.006**	**0.009**	**0.005**	**0.018**	**0.017**	**0.006**	**0.006**	**0.006**	**0.004**

Poznámky: OD – oculus dexter; OS – oculus sinister; vyšetření – klinické vyšetření 4.9[±0.6] měsíců, 25.0[±0.6] měsíců, a 49.9[±0.5] měsíců po propouštění z nemocnice (průměr a SD).

Chronický pokles tloušťky RNFL, dynamika ztráty zrakových funkcí a postižení mozku na magnetické rezonanci.

U pacientů se signifikantním poklesem tloušťky RNFL jsme zjistili progredující ztrátu zrakových funkcí v 7 z 10 případů. Progredující ztráta ostrosti zraku byla zjištěna u 4 pacientů, progrese výpadků zorného pole a zhoršení perimetru u 6 pacientů, pokles kontrastní citlivosti u 3 pacientů a zhoršení barvocitu u 5 pacientů. Všichni pacienti s progredující ztrátou zrakových funkcí byli muži ve věku od 33 do 69 let. Pouze pacient číslo 7 (viz Tabulku 9) měl diabetes mellitus 2. typu na metforminu. Nebyla zjištěna žádná další relevantní somatická nebo oční onemocnění, která by vysvětlovala pokles zrakových funkcí.

Známky poškození mozku na magnetické rezonanci byly přítomny u 18 ze 42 (43%) pacientů. Symetrická bilaterální nekrotická ložiska v putamen byla zjištěna u 16 pacientů. U 12 pacientů (67%), byly přítomny hemorrhagické léze v mozku. Patologické léze zrakového nervu na MRI byly zjištěny u třech pacientů. Výsledky druhého a třetího MRI vyšetření mozku neprokázaly známky progrese ani regrese zjištěných nálezů.

Byla přítomna pozitivní asociace mezi MRI známkami poškození mozku, přítomností hemorrhagických ložisek v mozku a poklesem tloušťky globální a temporální RNFL v období sledování (všechna $p < 0.05$). Prevalence dlouhodobých zrakových následků u pacientů s hemorrhagickými lézemi v mozku byla vyšší než u pacientů s nekrotickými ložisky v mozku bez hemorrhagických známek. Pacienti s abnormální tloušťkou RNFL měli MRI známky poškození mozku v 10 z 13 případů, pacienti s normální tloušťkou RNFL – pouze v 8 z 29 případů ($p = 0.003$). Hemorrhagické známky v mozku byly přítomny u 7 z 13 pacientů s abnormální tloušťkou RNFL a pouze u 5 z 29 pacientů s normální tloušťkou RNFL ($p = 0.015$).

Zjistili jsme, že akutní poškození gangliových buněk neuronů oční sítnice kyselinou mravenčí při otravě metanolem je následováno chronickou degenerací

Tabulka 9. Dynamika ztráty zrakových funkcí u pacientů se signifikantním poklesem tloušťky RNFL v průběhu 4 let sledování (n=10).

Pacient (OD/OS)	1.	2.	3.	4.**	5.	6.	7.	8.	9.**	10.
Věk/ Pohlaví	69/M	58/M	61/M	48/M	48/M	34/M	65/M	46/M	33/M	38/M
VA 1. vyšetření	1.0/0.3*	0.1/0	0.6/0.6	0.03/0.03	0.6/0.6	1.0/0.8	1.0/0.6	0.06/0.03	0.03/0.01	0.9/0.7
VA 2. vyšetření	1.0/1.0	0.1/0	06./0.7	0.03/0.03	0.6/0.5	0.9/0.8	1.0/0.8	0.1/0.06	0.08/0.03	0.6/0.6
VA 3. vyšetření	1.0/1.0	**0.08/0**	0.6/0.6	**FC/0.03**	1.0/0.7	0.9/0.9	**0.6/0.6**	0.15/0.3	0.08/0.03	**0.5/0.4**
Perimetr 1. vyšetření	Nazální dolní defekty OO	OD residua paracentrální horní / OS nejde	OD zúžení / OS normální	OD/OS zúžení v horním a dolním poli	OD/OS cirkulární zúžení	OD/OS nazální zúžení	OD/OS defekty v nazálním a temporálním dolním segmentech	OD/OS centrocekální scotomata	OD/OS defekty v celém zorném poli	normální
Perimetr 2. vyšetření	stejně	**Nejde**	OD zúžení / OS normální	stejně	**OS progrese k centru**	**OS progrese v temporálním segmentu**	stejně	stejně	**OD/OS residua v nazálním a v horním polích**	**OD/OS nízká spolehlivost**
Perimetr 3. vyšetření	stejně	**Nejde**	OD zúžení / OS normální	**OD/OS residua temporální a nazální**	stejně	stejně	stejně	stejně	stejně	stejně
CS 1. vyšetření	N/B*	P/ND	N/B	P/P	P/P	B/B	P/P	P/P	ND	B/P

CS 2. vyšetření	N/N	P/ND	N/B	**ND**	B/P	B/B	B/B	B/P	P/ND	B/P
CS 3. vyšetření	B*/N	P/ND	N/B	**ND**	P/P	**B/P**	B/B	P/P	P/ND	**P/P**
CV 1. vyšetření	N/N	P/ND	N/N	P/P	N/N	N/B	N/N	B/P	ND	N/N
CV 2. vyšetření	B/N	P/ND	N/B	**ND**	N/N	N/N	N/B	B/B	ND	N/N
CV 3. vyšetření	B/B	P/ND	N/N	**ND**	**N/P**	**B/B**	**N/B**	B/B	P/ND	**B/P**

*Poznámky: OD – oculus dexter; OS – oculus sinister; VA – ostrost zraku; CS – kontrastní citlivost; CV – barvocit; FC – počet prstů; ND – nedetekovatelný/neměřitelný; P – patologický nález; B – hraniční nález; N – normální nález; M – muž. Tučné písmo ukazuje progresi. * - katarakta; ** - v důsledku špatného centrálního vizu výsledky dalších vyšetření mají nízkou validitu.*

a progredující ztrátou axonů prakticky u 25 % pacientů přeživších otravu. Tento proces je doprovázen progredující ztrátou zrakových funkcí. Většina pacientů s abnormální tloušťkou RNFL měla známky poškození mozku na MRI. Byla přítomna významná asociace mezi vstupním pH arteriální krve pacientů při příjmu do nemocnice a stupněm akutního poškození oční sítnice, jakož i rychlosti chronické ztráty axonů zrakového nervu. Přednemocniční aplikace etanolu a vyšší rychlost eliminace a korekce acidemie za intermitentní hemodialýzy odpovídaly větší tloušťce RNFL u pacientů ze sledovaného souboru. Naše výsledky ukazují, že tloušťka globální a temporální RNFL, ne však v nazálních segmentech, byla ve sledovaném souboru signifikantně nižší než u kontrolního souboru stejného věku, pohlaví a se stejným podílem osob s chronickým alkoholismem. Zjistili jsme, že rychlost poklesu tloušťky RNFL v důsledku ztráty neuronálních axonů u pacientů přeživších těžkou otravu metanolem se závažnou acidemií je signifikantně vyšší než rychlost fyziologického poklesu spojeného se stárnutím. Skutečnost, že nervová vlákna sítnice v temporálních segmentech s menším průměrem axonů byla více vulnerabilní než vlákna z nazálních segmentů s více heterogenní populací axonů, která zůstávala intaktní, ukazuje na možný podíl mitochondriální dysfunkce na procesu chronické neurodegenerace po optické neuropatii způsobené metanolem (Sadun, 1998; Pan et al, 2012). U jiné optické neuropatie se známým podílem mitochondriální dysfunkce na patogenezi onemocnění, Leberové hereditární optické neuropatii, pořadí postižených segmentů oční sítnice začíná temporálními segmenty jako prvními a končí nazálními jako posledními (Barboni et al., 2010).

Toxicita kyseliny mravenčí má přímý vliv na mitochondriální funkci: inhibice cytochromu c oxidázy vede k tvorbě velice agresivního prostředí reaktivních sloučenin kyslíku (reactive oxygen species, ROS) ve fázi akutní otravy metanolem (Liesivuori et al., 1991; Du, 2012). Mitochondriální dysfunkce vede ke zvýšené produkci ROS a toxických aldehydů, oxidativnímu poškození buněčných

struktur včetně mitochondriálních membrán, uvolnění cytochromu c z intermembránového prostoru a indukci buněčné apoptózy (Yang et al., 1997). Primární poškození způsobuje neuronální degeneraci, apoptózu a smrt nejmenších nervových vláken, avšak další menší nervová vlákna, která primární poškození přežila, mohou obsahovat mitochondrie se získanou poruchou v důsledku vysokého počtu akumulovaných delecí mitochondriální DNA (mtDNA) vzniklých při akutním stavu působením ROS ve vysokých koncentracích. Mitochondriální DNA je velice citlivá vůči oxidačnímu stresu v důsledku působení ROS (Hollensworth et al, 2000). Persistence poškozené mtDNA vede k mutacím v mitochondriálním genomu a k další mitochondriální dysfunkci, která vyvolává opožděnou reakci chronické neurodegenerace pozorovanou v naší studii. Jiné příčiny toho, že tento proces pokračuje i dlouho po tom, co akutní poškození odeznělo, mohou být vysvětleny účinkem "společného rezervoáru" popsaného Pan et al. (2012): rozšiřování signálních molekul pro indukci apoptózy, uvolnění excitotoxických neurotransmiterů a redukce trofické zpětné vazby pro buňky glie se ztrátou gangliových buněk neuronů sítnice jsou možnými prvky zprostředkujícími tento fenomén.

Závažnost acidemie při příjmu do nemocnice korelovala jak s tloušťkou globální a temporální RNFL, tak i s rychlostí ztráty axonů zrakového nervu. Konstanta disociace kyseliny mravenčí (pKa) je 3.8, tedy pokles pH arteriální krve o 0.3 vede ke zdvojnásobení nedisociované složky kyseliny mravenčí a značnému zvýšení její neurotoxicity, protože pouze nedisociovaná kyselina mravenčí je schopna přestupovat neuronální membránou (Liesivuori et al., 1991; Zakharov et al., 2016). Vyšší rychlost eliminace kyseliny mravenčí a korekce acidemie při aplikaci intermitentní hemodialýzy v porovnání s kontinuálními modalitami může

být příčinou lepšího stavu sítnice u pacientů léčených touto modalitou dialýzy (Zakharov et al., 2014; 2017).

Chronická ztráta axonů sítnice může být způsobena jinými patologickými stavy nesouvisejícími s akutní otravou metanolem. Deficit vitamínů B_{12} nebo B_1 v důsledku chronického alkoholismu nebo nutričního deficitu, hypofunkce štítné žlázy nebo hyperglykemie v důsledku nedostatečně kompenzovaného diabetes mellitus mohou vést ke ztrátě axonů zrakového nervu v důsledku neadekvátních trofických a ochranných funkcí glie (Misra et al., 2003; Fei et al., 2008; Yeh et al., 2013; Fernandez et al., 2004; Veselinovic et al., 2005). Ve sledovaném souboru nebyla přítomna asociace mezi koncentrací glukózy, TSH, vitamínů B_{12} a B_1 měřených opakovaně v době sledování a dynamikou ztráty axonů sítnice. Pouze jeden pacient s chronickou ztrátou axonů měl diabetes mellitus druhého typu. Žádný z pacientů neměl hypovitamínózu B_1, B_{12}, nebo sníženou funkci štítné žlázy.

V literatuře se diskutuje o asociaci mezi morfologickými změnami oční sítnice a změnami funkcí zrakového nervu registrovanými pomocí evokovaných zrakových potenciálů. Jsou studie demonstrující signifikantní asociaci mezi výsledky měření VEP a RNFL, jakož i studie tuto souvislost zpochybňující (Yiannikas et al., 1983; Fatehi et al., 2012; Klistorner et al., 2008; 2010). Naše výsledky ukazují, že míra asociace záleží na době měření. Progredující axonální degenerace vede časem k nárůstu asociace mezi tloušťkou RNFL a amplitudou N1P1 evokovaných zrakových potenciálů. Zajímavou skutečností byla asociace mezi amplitudou evokovaného komplexu a rychlostí ztráty axonů: čím byla amplituda N1P1 nižší při prvním měření, tedy čím byl větší počet akutně poškozených gangliových buněk sítnice, tím vyšší byla rychlost chronické axonální degenerace v následujících letech. Progrese ztráty zrakových funkcí u většiny pacientů s chronickou degenerací neuronů oční sítnice zjištěná v rámci naší studie

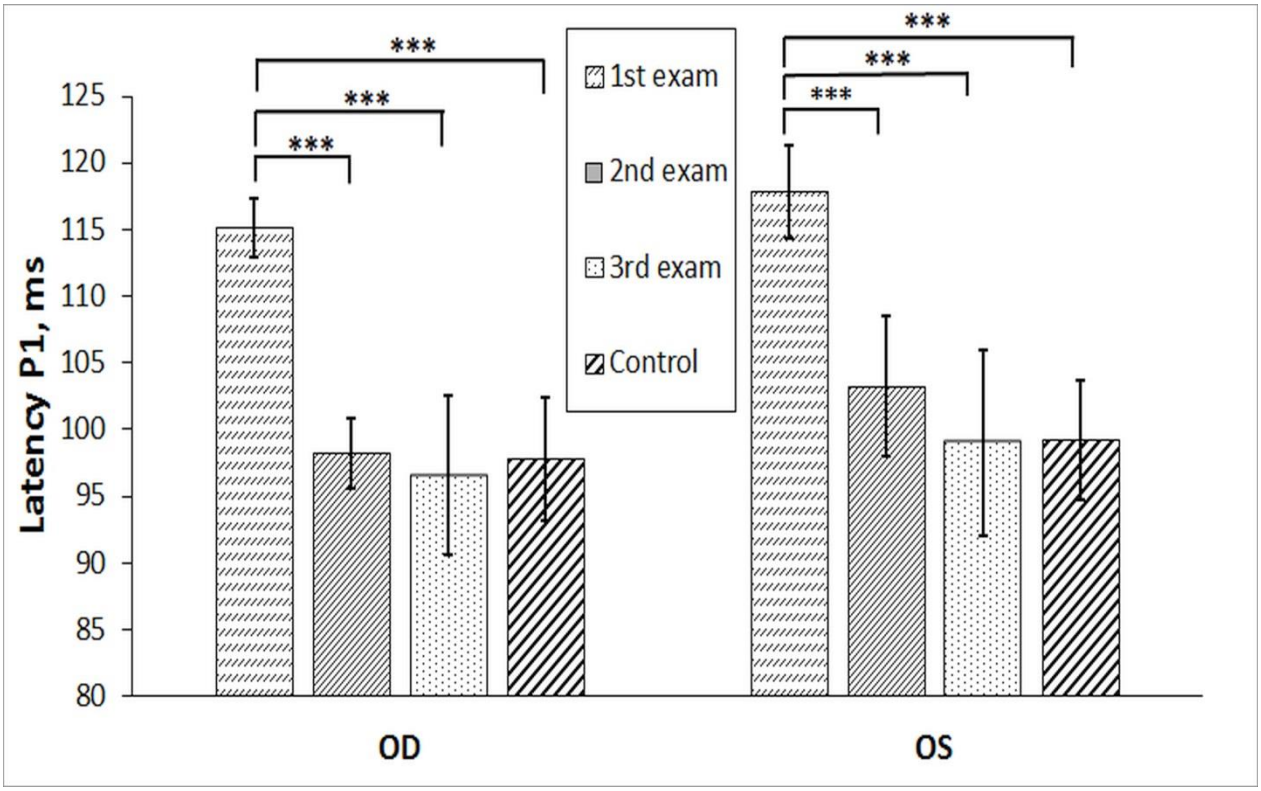
představuje závažný problém z hlediska kvality života pacientů v letech po propouštění z nemocnice (Langelaan et al., 2007; Rulisek et al., 2017). U 10 z 13 pacientů s chronickou ztrátou axonů zrakového nervu jsme zaznamenali další pokles zrakových funkcí, který pacienty limitoval v běžném a profesním životě: pokles ostrosti zraku, kontrastní citlivosti, výpadky a zúžení zorného pole, zhoršení barvocitu. Atrofie disku zrakového nervu měla difuzní charakter, výraznější v temporálních segmentech, bez známek exkavace charakteristických pro glaukom. Výpadky zorného pole také nebyly charakteristické pro glaukom. Nejčastěji byly lokalizovány v horních a nazálních periferních polích, nálezy byly bilaterální a většinou asymetrické. V jednom případě jsme registrovali centrocekální skotom, ale u většiny sledovaného souboru centrální vízus postižen nebyl (s výjimkou nejzávažnějších případu otravy). Jiné příčiny progrese ztráty zrakových funkcí somatického charakteru nebo jiná oční onemocnění u pacientů ze sledovaného souboru byly vyloučeny. Tato skutečnost zdůrazňuje nezbytnost dispenzarizace a pravidelných oftalmologických kontrol u pacientů přeživších akutní otravu metanolem. Asociace mezi chronickou neurodegenerací sítnice a MRI známkami poškození mozku svědčí o možné souvislosti mezi zrakovými a neurologickými následky otravy. MRI mozku není rutinním vyšetřením u všech pacientů s akutní otravou metanolem a podíl včas nerozpoznaných lézí mozku je vysoký (Zakharov et al., 2016; Vaneckova et al., 2014, 2016). Tedy část pacientů s včas nerozpoznaným poškozením bazálních ganglií může mít v průběhu následujících let sekundární parkinsonismus bez zjištění příčinné souvislosti s otravou metanolem v minulosti. Známky progredující neurodegenerace sítnice vykazují významnou asociaci se známkami nekrotických hemorrhagických lézí mozku, což potvrzuje nutnost MRI vyšetření mozku a periodických neurologických kontrol u pacientů z této rizikové skupiny.

5.3. Klinické, biochemické a genetické determinanty chronických změn zrakových funkcí v průběhu čtyř let po akutní optické neuropatii způsobené intoxikací metanolem (Publikace IV, V)

Akutní demyelinizace zrakového nervu v důsledku přímého toxického účinku kyseliny mravenčí může vést k axonální degeneraci v důsledku selhání trofické funkce a přerušení normální interakce mezi axonem a myelinem. Studie akutní optické neuritidy u pacientů s roztroušenou sklerózou demonstrují možnost spontánní remyelinizace axonů zrakového nervu, která se projevuje zkrácením latence vlny P1 evokovaného potenciálu (Brusa et al., 1999; 2001). Výsledky opakovaného měření VEP v rámci longitudinální studie mohou poskytnout důležité informace o chronických změnách konduktivity zrakového nervu a jejich dynamice spojených s reparací myelinu a obnovením integrity zrakových cest. Prodloužení latence vlny P1 odráží stupeň demyelinizace axonů zrakového nervu a pokles amplitudy N1P1 evokovaného potenciálu indikuje počet funkčních axonů po jejich akutním poškození v důsledku různých příčin (Jones et al., 2003). V rámci prospektivní longitudinální kohortové studie jsme sledovali dynamiku, klinické, biochemické a genetické (polymorfismus apolipoproteinu E) determinanty chronických změn funkcí zrakových cest v průběhu 4 let následujících po akutní intoxikaci metanolem ve sledovaném souboru pacientů přeživších akutní intoxikaci metanolem.

Dynamika latence vlny P1 evokovaného potenciálu v době sledování

Výsledky měření latence vlny P1 zrakového evokovaného potenciálu v průběhu 4 let sledování jsou uvedeny na obrázku 9. Při prvním vyšetření abnormální latence vlny P1 byla zjištěna na 18/42 (43 %) pravých očí a na 21/42 (50%) levých očí, včetně 5 OD a 4 OS s nevybavitelným evokovaným potenciálem.

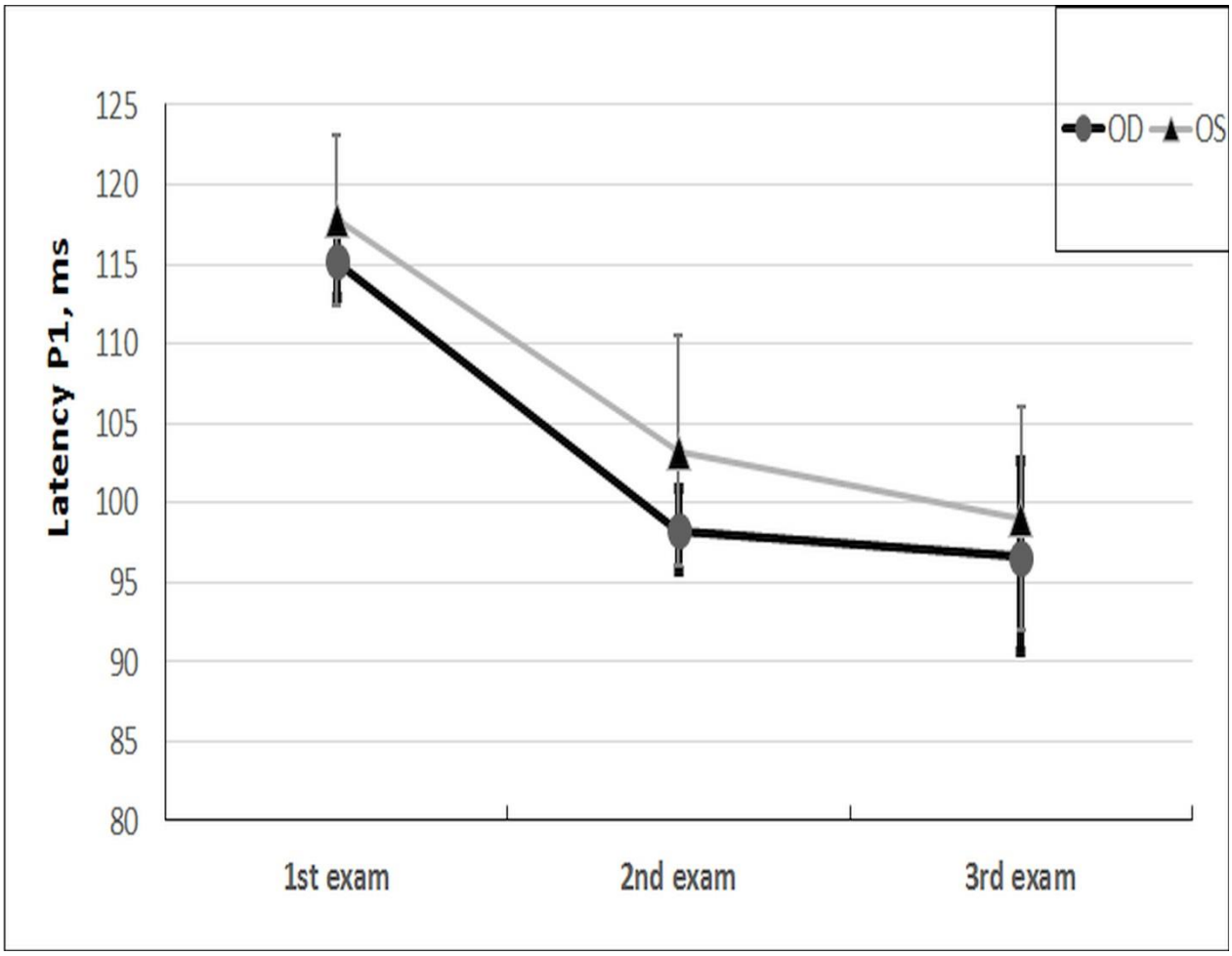


Obrázek 9. Latence vlny P1 zrakových evokovaných potenciálů ve sledovaném souboru (n=42) versus v kontrolním souboru (n=41).

*Poznámky: OD – oculus dexter; OS – oculus sinister; ms – milisekundy; exam – klinické vyšetření 4.9 ± 0.6 měsíců (první), 25.0 ± 0.6 měsíců (druhé), a 49.9 ± 0.5 měsíců (třetí) po propouštění z nemocnice (průměr a SD); *** - p<0.001; ** - p<0.01; * - p<0.05.*

Ve sledovaném souboru bylo zaznamenáno signifikantní zkrácení průměrné latence P1 s největším poklesem v období mezi prvním a druhým kolem vyšetření, tedy do dvou let od akutní toxické optické neuropatie, s návratem prodloužené latence P1 k hodnotám stejným jako v kontrolním souboru (Obrázek 9).

Průměrné zkrácení latence P1 ve sledovaném souboru bylo 15.0 ± 2.0 ms pro 36/42 (86 %) OD a 14.9 ± 2.4 ms pro 35/42 (83 %) OS, s maximálním zkrácením u některých pacientů o 29.0 až 35.0 ms (Obrázek 10).



Obrázek 10. Dynamika změn průměrné latence vlny P1 ve sledovaném souboru během 4 let po akutní otravě metanolem.

*Poznámky: OD – oculus dexter; OS – oculus sinister; ms – milisekundy; exam – klinické vyšetření 4.9 ± 0.6 měsíců (první), 25.0 ± 0.6 měsíců (druhé), a 49.9 ± 0.5 měsíců (třetí) po propouštění z nemocnice (průměr a SD); *** - p<0.001; ** - p<0.01; * - p<0.05.*

Nicméně, u 5 OD a 4 OS zrakový potenciál zůstal nevybavitelný a u dalšího 1 OD a 3 OS původně vybavitelný zrakový potenciál se stal nevybavitelným při druhém nebo třetím vyšetření. Negativní dynamika a nevybavitelný zrakový potenciál byly registrovány u pacientů s těžkou otravou a závažnou acidemií při příjmu do nemocnice. Tito pacienti měli nedetekovatelný etanol v krevním séru,

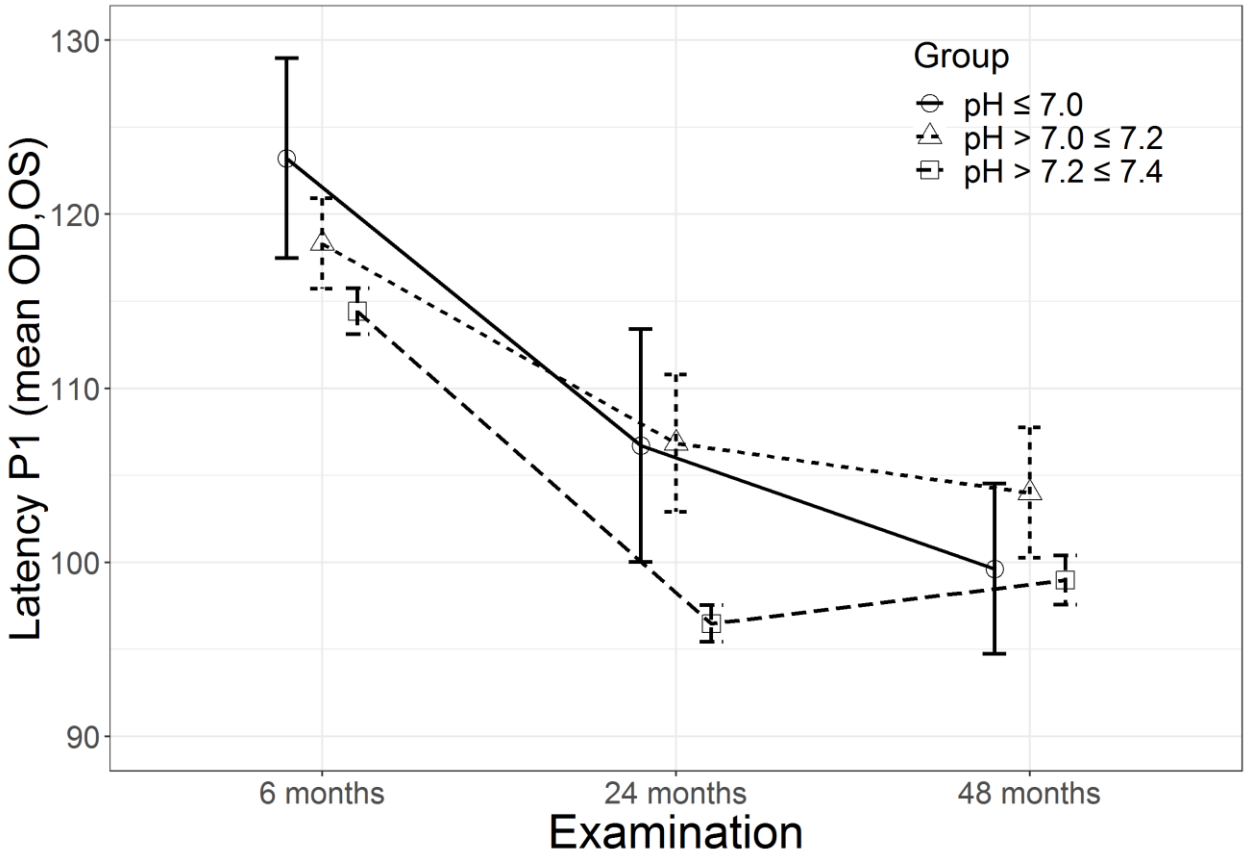
vysoké koncentrace kyseliny mravenčí, laktátu, kreatininu a nízké pH arteriální krve.

Byla zjištěna signifikantní asociace mezi pH arteriální krve při příjmu do nemocnice, jako ukazatelem závažnosti metabolické acidózy, a latencí vlny P1 měřenou při prvním a druhém vyšetření ($r_1 = –0.349$; $p = 0.040$, a $r_2 = –0.379$; $p = 0.020$), avšak ne při třetím vyšetření, kdy remyelinizace axonu zrakového nervu již byla dokončena. Stejná asociace byla přítomna mezi koncentrací metanolu v séru a latencí vlny P1: $r_1 = 0.324$; $p = 0.047$, $r_2 = 0.526$; $p < 0.001$ a $r_3 = 0.141$; $p = 0.410$. Konečně, byla přítomna pozitivní asociace mezi latencí P1 měřenou při prvním a druhém vyšetření a věkem pacientů ($r_1 = 0.509$; $p = 0.002$, $r_2 = 0.494$; $p = 0.003$). Nebyla přítomna asociace mezi latencí P1 ani rychlostí zkrácení latence a pohlavím nebo laboratorními parametry měřenými ve sledovaném období (glukóza, cholesterol, triacylglyceridy, vitamíny B_1, B_{12}, TSH, aj.). Avšak rychlost zkrácení latence P1 byla pomalejší u pacientů s vyšší koncentrací enzymu gamma glutamyl transferázy, GGT ($r = 0.359$; $p = 0.030$).

Druh antidota aplikovaného v nemocnice (etanol nebo 4-methylpyrazol) nebyl pro latenci P1 signifikantní. Pacienti, u kterých byla aplikována kontinuální hemodialýza, měli prodlouženou latenci P1 v porovnání s pacienty s intermitentní hemodialýzou při prvním a druhém vyšetření ($r_1 = 0.343$; $p = 0.035$ a $r_2 = 0.342$; $p = 0.040$). Efekt substituce folátů na dynamiku latence P1 byl nevýznamný.

Pro multivariántní analýzu dynamiky změn latence vlny P1 ve sledovaném období byly zvoleny následující nezávislé proměnné jako prediktory: věk, pohlaví, délka časového intervalu od požití metanolu do začátku hospitalizace, rozdíl OD / OS v latenci P1, akutní laboratorní parametry měřené při příjmu do nemocnice (pH arteriální krve, koncentrace metanolu, etanolu a glukózy v séru), koncentrace vitamínů B_1 a B_{12}, TSH, měřené v době sledování. Výsledky multivariántní analýzy

proměnných významných pro dynamiku změn latence vlny P1 jsou uvedeny v tabulce 10. Prognostický model předpovídající změny latence P1 v průběhu 4 let po akutní toxické optické neuropatii v závislosti na pH arteriální krve při příjmu do nemocnice po adjustaci na další nezávislé proměnné je uveden na obrázku 11. Porovnání predikovaných dat z hlediska dynamiky latence P1 s faktickými daty získanými na základě měření ve sledovaném souboru je uvedeno na obrázku 12.



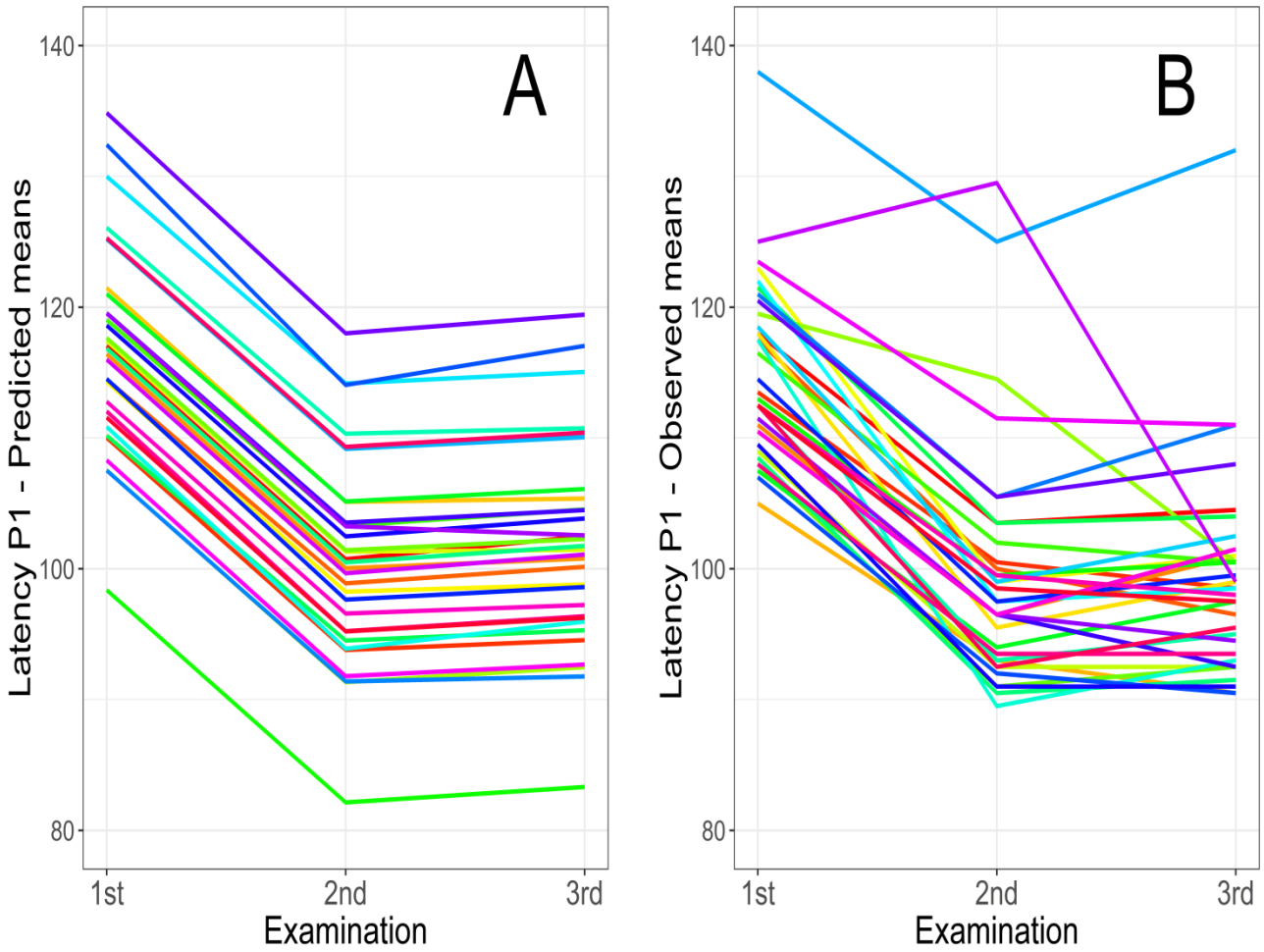
Obrázek 11. Model předpovídající dynamiku změn latence P1 v průběhu 4 let po akutní otravě metanolem v závislosti na pH arteriální krve při příjmu do nemocnice.

Poznámky: Latency P1 – průměrná latence P1, ms; pH – pH arteriální krve; examination – klinické vyšetření 4.9 ± 0.6 měsíců (první), 25.0 ± 0.6 měsíců (druhé), a 49.9 ± 0.5 měsíců (třetí) po propouštění z nemocnice (průměr a SD).

Tabulka 10. Regresní analýza nezávislých proměnných významných pro chronické změny latence vlny P1 v průběhu 4 let sledování.

Proměnná	Odhad	Standardní odchylka	df	t	Signifikance	95% Konfidenční Interval	
						Dolní hranice	Horní hranice
Intercept	262.87	68.89	22.13	3.82	**0.00**	120.06	405.69
Doba vyšetření	-12.43	0.53	72.95	-23.27	**0.00**	-13.49	-11.36
$Čas^2$	2.14	0.12	58.51	17.70	**0.00**	1.90	2.38
OD/OS rozdíl	-0.99	0.38	43.69	-2.66	**0.01**	-1.75	-0.24
Pohlaví	3.21	2.04	20.98	1.57	0.13	-1.04	7.46
Věk	0.41	0.12	21.96	3.31	**0.00**	0.15	0.66
pH	-24.54	9.35	21.99	-2.63	**0.02**	-43.92	-5.16
MetOH	0.00	0.00	21.51	-1.31	0.21	-0.01	0.00
EtOH	0.00	0.00	22.61	1.22	0.24	0.00	0.01
Glukóza	0.00	0.12	21.49	0.02	0.98	-0.24	0.24
B_1_1.	0.26	0.12	22.27	2.12	**0.05**	0.01	0.51
B_1_2.	-1.11	0.69	78.53	-1.62	0.11	-2.48	0.26
TSH	0.05	0.15	66.64	0.35	0.73	-0.24	0.34
B_{12}_1.	0.00	0.00	22.01	-0.09	0.93	-0.01	0.00
B_{12}_2.	0.01	0.02	21.33	0.50	0.62	-0.03	0.05
B_{12}_3.	0.00	0.02	21.19	-0.08	0.94	-0.04	0.04

Poznámky: Doba vyšetření – doba klinického vyšetření (t_1 - 4.9[±0.6] měsíců, t_2 - 25.0[±0.6] měsíců, a t_3 - 49.9[±0.5] měsíců po propouštění z nemocnice; $Čas^2$ – délka časového intervalu od požití metanolu do začátku hospitalizace (nelineární trend je zohledněn druhou mocninou); OD/OS rozdíl – interokulární rozdíl v průměrné latenci P1; pH – pH arteriální krve při příjmu; MetOH – koncentrace metanolu; EtOH – koncentrace etanolu; Glukóza – koncentrace glukózy; B1 – koncentrace vitamínu B_1; B_{12} – koncentrace vitamínu B_{12}; TSH – koncentrace thyreotropního hormonu; 1., 2., 3. - jednotlivá měření 4.9[±0.6] měsíců, 25.0[±0.6] měsíců, a 49.9[±0.5] měsíců po propouštění z nemocnice (průměr a SD).



Obrázek 12. Porovnání modelové predikce (A) a faktických dat (B) dynamiky remyelinizace zrakového nervu v průběhu 4 let po akutní intoxikaci metanolem.

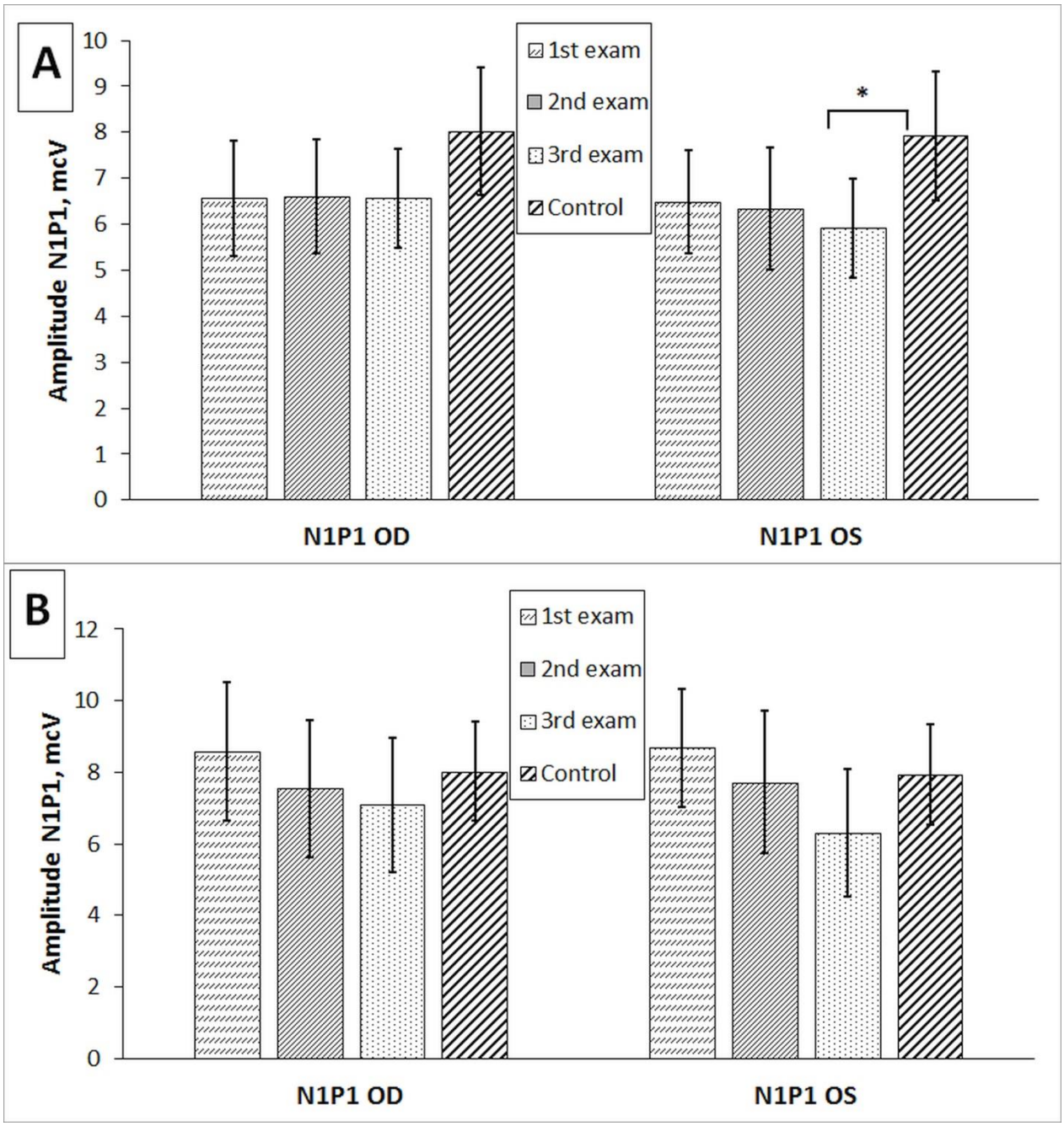
Poznámky: „Predicted means" - Průměrné predikované hodnoty – průměrné hodnoty latence P1 předpovídané modelem pro jednotlivce ze sledovaného souboru; „Observed means" – Průměrné sledované hodnoty – průměrné hodnoty latence P1 měřené v době sledování u jednotlivců ze sledovaného souboru.

Dynamika změn amplitudy N1P1 evokovaného potenciálu v době sledování

Výsledky třech měření amplitudy N1P1 jsou uvedeny na obrázku 13. Při prvním měření byla abnormální amplituda N1P1 zjištěna u 10 / 42 (24 %) OD a 10 / 42 (24 %) OS, včetně 5 OD a 4 OS bez vybavitelné odpovědi. Při třetím měření byla abnormální amplituda N1P1 zjištěna u 10 / 42 (24 %) OD a 14 / 42 (33 %) OS, včetně 6 OD a 7 OS bez vybavitelné odpovědi. Na jednom 1 OD a 3 OS s abnormální amplitudou N1P1 měřenou při prvním vyšetření se stal evokovaný potenciál nevybavitelným při druhém nebo třetím vyšetření.

Průměrná amplituda N1P1 ve sledovaném souboru byla nesignifikantně nižší než v kontrolním souboru (1. vyšetření: 6.6 ± 1.2 versus 8.0 ± 1.4 (OD); 6.5 ± 1.1 versus 7.9 ± 1.4 mcV (OS); 2. vyšetření: 6.6 ± 1.2 versus 8.0 ± 1.4 (OD); 6.3 ± 1.3 versus 7.9 ± 1.4 mcV (OS); 3. vyšetření: 6.5 ± 1.1 versus 8.0 ± 1.4 (OD); 5.9 ± 1.1 versus 7.9 ± 1.4 mcV (OS); všechna $p > 0.05$). V době sledování byly pozorovány pouze nesignifikantní změny průměrné amplitudy N1P1 ve sledovaném souboru (obrázek 5A). Průměrný pokles amplitudy byl –0.06 ± 0.56 mcV pro OD a –0.83 ± 0.64 mcV pro OS.

U 17 ze 36 (47 %) pacientů s měřitelnou amplitudou N1P1 byl v průběhu sledování pokles amplitudy o 1.0 mcV a více registrován aspoň na jednom oku (obrázek 5B). Průměrný pokles amplitudy N1P1 v této skupině (n=17) byl –1.11 ± 0.83 mcV pro OD a –2.37 ± 0.66 mcV pro OS, obě hodnoty byly signifikantně vyšší než hodnoty v celém sledovaném souboru ($p < 0.001$). U 8 ze 17 pacientů byl pokles amplitudy N1P1 o 1.0 mcV a více registrován na obou očích, u 9 pacientů pouze na jednom oku, většinou levém. Největší pokles byl 4.6 mcV v průběhu 4 let pro pravé oko a 6.6 mcV v průběhu 4 let pro levé oko. Žádný z pacientů se signifikantním poklesem amplitudy N1P1 neměl abnormální hodnoty



Obrázek 13. Výsledky měření amplitudy N1P1 evokovaného potenciálu v celém sledovaném souboru (A, n=42) a u pacientů se signifikantním poklesem amplitudy (B, n=17) v porovnání s kontrolní skupinou (n=41).

*Poznámky: OD – oculus dexter; OS – oculus sinister; exam – klinické vyšetření 4.9 ± 0.6 měsíců (první), 25.0 ± 0.6 měsíců (druhé), a 49.9 ± 0.5 měsíců (třetí) po propouštění z nemocnice (průměr a SD); *** - p<0.001; ** - p<0.01; * - p<0.05.*

amplitudy při prvním vyšetření a pouze 3 / 17 měli abnormální hodnoty při druhém nebo třetím vyšetření. Ostatní pacienti (14 ze 17) měli normální hodnoty amplitudy N1P1 ve sledovaném období přes registrovaný signifikantní pokles o 1.0 mcV a více.

Asociace mezi amplitudou N1P1 a latencí P1 evokovaných potenciálů byla nejvýznamnější při prvním vyšetření, méně významná ale signifikantní při druhém vyšetření, konečně nesignifikantní při třetím vyšetření. Věk pacientů negativně koreloval s amplitudou N1P1 v průběhu celého období sledování (pro OD / OS: r_1 = –0.469 / –0.474; p = 0.004 / 0.003; r_2 = –0.436 / –0.448; p = 0.009 / 0.006; r_3 = –0.454 / –0.422; p = 0.005 / 0.010).

Pacienti s nižší amplitudou N1P1 měli těžší otravu a nižší Glasgow coma scale, GCS (r = 0.418; p = 0.009), vyšší koncentraci metanolu (r = –0.414; p = 0.010) při příjmu do nemocnice. Vliv druhu použitého antidota a substituce foláty na amplitudu N1P1 byl nesignifikantní. Pacienti, u kterých byla aplikována kontinuální hemodialýza, měli nižší amplitudu N1P1 v porovnání s pacienty léčenými pomoci intermitentní hemodialýzou (r_1 = –0.495; p = 0.002, r_2 = –0.382; p = 0.021).

Chronické strukturní a funkční změny ve zrakovém systému a role polymorfismu genu ApoE

Pro analýzu možného vlivu polymorfismu genu ApoE na dynamiku změn zrakových funkcí bylo provedeno vyšetření genotypu ApoE u 49 pacientů (98 očí) přeživších akutní otravu metanolem, včetně 41 pacientů (82 očí) se všemi třemi klinickými vyšetřeními, ze sledovaného souboru. Frekvence jednotlivých genotypů ApoE ve sledovaném souboru se nelišila od frekvence genotypů v běžné České populaci (Tabulka 11).

Tabulka 11. Distribuce genotypu a alel apolipoproteinu E ve sledovaném souboru a v běžné České populaci.*

Genotyp	Pacienti		Kontroly		P
	n	%	n	%	
ApoE2/E2	1	2.0	42	0.7	0.25
ApoE3/E2	8	16.3	708	11.4	0.46
ApoE3/E3	28	57.1	4126	66.3	0.26
ApoE4/E3	10	20.4	1155	18.5	0.20
ApoE4/E4	2	4.1	77	1.2	0.08
ApoE4/E2	0	0.0	122	2.0	--

*Poznámky: * - data ze studie Hubacek et al., 2013.*
E2, E3, E4 – různé alely genu kódujícího apolipoprotein E.
Rozdíl $p<0.05$ byl považován za signifikantní.

Asociace genotypu ApoE s chronickými změnami funkcí zrakového nervu a morfologického stavu sítnice (RNFL) je uvedena v tabulce 12. Pacienti s alelou ApoE4 měli menší tloušťku globální a temporální RNFL při všech třech vyšetřeních v porovnání s pacienty bez ApoE4 alely. Dále, pacienti s ApoE4 alelou měli prodlouženou latenci P1 při prvním vyšetření (předtím než proběhla remyelinizace axonů zrakového nervu) v porovnání s pacienty bez ApoE4 alely (tabulka 13). Z pěti pacientů s nevybavitelným zrakovým evokovaným potenciálem aspoň na jednom oku, čtyři pacienti byli nosiči ApoE4 alely. Ve sledovaném souboru pacientů, odds ratio (95% CI) pro abnormální nález VEP při prvním vyšetření (prodloužená latence P1 a/nebo abnormální amplituda N1P1 nebo nevybavitelná odpověď) bylo 8.92 (3.00 – 36.50) pro nosiče ApoE4 alely v porovnání s pacienty bez ApoE4 alely ($p < 0.001$).

Byla zjištěna signifikantní asociace mezi přítomností ApoE4 alely a přítomností nekrotických lézí v mozku na MRI ($r = 0.384$; $p = 0.013$), jakož i hemorrhagických lézí v důsledku intoxikace metanolem ($r = 0.395$; $p = 0.011$). Nebyla zjištěna asociace mezi polymorfismem ApoE a pohlavím, věkem, akutní koncentrací metanolu, etanolu, kyseliny mravenčí, jakož i léčebnými modalitami (druh antidota, hemodialýzy, substituce folátů, aj.).

V rámci studie jsme zjistili, že úplné nebo částečné obnovení konduktivity zrakového nervu po akutním poškození myelinového obalu nastává u více než 80 % pacientů v průběhu 4 let v důsledku remyelinizace axonů zrakového nervu, přičemž nejvyšší rychlost tohoto procesu byla zaznamenána v průběhu prvních dvou let po propouštění z nemocnice. Délka časového intervalu od požití metanolu do začátku hospitalizace, závažnost acidemie a věk pacienta byly nejvýznamnějšími nezávislými proměnnými, které měly vliv na dynamiku obnovení konduktivity zrakového nervu.

Tabulka 12. Asociace mezi skupinou ApoE genotypu (ApoE2/E2(E3/E2) versus ApoE3/E3 versus ApoE4/E3(E4/E4)) s chronickými morfologickými a funkčními změnami zrakových cest v době sledování (n=41).

Parametr	**Průměr ± SD**	**R**	**P**
RNFL OD globální, 1st vyšetření, µm	88.3±5.5	-0.418	**0.007**
RNFL OD globální, 2nd vyšetření, µm	84.6±6.9	-0.466	**0.002**
RNFL OD globální, 3rd vyšetření, µm	83.3±7.1	-0.458	**0.003**
RNFL OS globální, 1st vyšetření, µm	84.9±6.4	-0.430	**0.006**
RNFL OS globální, 2nd vyšetření, µm	81.9±7.6	-0.463	**0.002**
RNFL OS globální, 3rd vyšetření, µm	80.4±7.7	-0.455	**0.003**
RNFL OD temporální, 1st vyšetření, µm	59.9±5.3	-0.421	**0.006**
RNFL OD temporální, 2nd vyšetření, µm	57.7±6.0	-0.457	**0.003**
RNFL OD temporální, 3rd vyšetření, µm	56.8±5.9	-0.470	**0.002**
RNFL OS temporální, 1st vyšetření, µm	55.1±4.9	-0.353	**0.026**
RNFL OS temporální, 2nd vyšetření, µm	53.6±5.7	-0.377	**0.015**
RNFL OS temporální, 3rd vyšetření, µm	53.6±5.8	-0.387	**0.012**
Latence P1 OD, 1st vyšetření, ms	115.2±2.2	0.362	**0.030**
Latence P1 OS, 1st vyšetření, ms	117.8±3.5	0.373	**0.021**
Latence P1 OD, 2nd vyšetření, ms	98.3±2.6	0.409	**0.015**
Latence P1 OS, 2nd vyšetření, ms	103.3±5.3	0.303	0.073
Latence P1 OD, 3rd vyšetření, ms	99.3±2.6	-0.141	0.405
Latence P1 OS, 3rd vyšetření, ms	101.8±4.1	-0.034	0.843

Poznámky: RNFL – tloušťka vrstvy nervových vláken sítnice; OD – oculus dexter; OS – oculus sinister; vyšetření – klinické vyšetření 4.9±0.6 měsíců, 25.0±0.6 měsíců, and 49.9±0.5 měsíců po propouštění z nemocnice. Rozdíl p<0.05 byl považován za signifikantní.

Tabulka 13. Chronické morfologické a funkční změny zrakových cest v době sledování ve skupině nositelů alely ApoE4 v porovnání se skupinou bez alely ApoE4 ve sledovaném souboru (n=41).

	ApoE4 nositelé (n=11)	**Bez ApoE4 alely** (n=30)	**P**
RNFL OD globální, 1st vyšetření, µm	76.0±16.0	92.4±5.0	0.053
RNFL OD globální, 2nd vyšetření, µm	68.0±20.0	90.6±5.7	**0.034**
RNFL OD globální, 3rd vyšetření, µm	65.0±20.0	89.6±6.0	**0.026**
RNFL OS globální, 1st vyšetření, µm	72.0±17.0	89.3±6.1	0.065
RNFL OS globální, 2nd vyšetření, µm	64.0±20.0	88.4±6.8	**0.030**
RNFL OS globální, 3rd vyšetření, µm	62.0±20.0	86.8±7.2	**0.029**
RNFL OD temporální, 1st vyšetření, µm	46.0±11.0	64.2±5.5	**0.003**
RNFL OD temporální, 2nd vyšetření, µm	42.0±12.0	63.3±6.1	**0.001**
RNFL OD temporální, 3rd vyšetření, µm	40.0±12.0	62.5±5.9	**0.001**
RNFL OS temporální, 1st vyšetření, µm	43.6±8.6	58.9±5.4	**0.006**
RNFL OS temporální, 2nd vyšetření, µm	40.0±11.0	58.5±6.2	**0.003**
RNFL OS temporální, 3rd vyšetření, µm	39.0±10.0	58.8±6.3	**0.00**
Latence P1 OD, 1st vyšetření, ms	120.1±3.7	114.0±2.4	**0.009**
Latence P1 OS, 1st vyšetření, ms	103.8±5.6	97.1±2.9	0.053
Latence P1 OD, 2nd vyšetření, ms	89.0±30.0	98.7±3.2	0.502
Latence P1 OS, 2nd vyšetření, ms	124.6±9.8	115.7±3.5	**0.033**
Latence P1 OD, 3rd vyšetření, ms	109.4±9.5	101.8±6.3	0.164
Latence P1 OS, 3rd vyšetření, ms	94.0±32.0	100.5±4.8	0.661

Poznámky: RNFL – tloušťka vrstvy nervových vláken sítnice; OD – oculus dexter; OS – oculus sinister; vyšetření – klinické vyšetření 4.9±0.6 měsíců, 25.0±0.6 měsíců, and 49.9±0.5 měsíců po propouštění z nemocnice. Rozdíl $p<0.05$ byl považován za signifikantní.

Amplituda evokovaného zrakového potenciálu, abnormální přibližně u každého čtvrtého pacienta ze sledovaného souboru při propuštění z nemocnice, odrážela tendenci k dalšímu poklesu v následujících letech. U poloviny pacientů s vybavitelným evokovaným potenciálem byl zaznamenán pokles amplitudy N1P1 o 1.0 mcV a více v době sledování; ani jeden z těchto pacientů neměl abnormální amplitudu evokovaného potenciálu při propuštění z nemocnice. U 2–7 % vyšetřených očí vedla progrese změn k dalšímu zhoršení funkcí zrakového nervu, od abnormální ale měřitelné amplitudy N1P1 k nevybavitelnému potenciálu. Zjistili jsme asociaci mezi polymorfismem genotypu ApoE a chronickými morfologickými a funkčními změnami zrakového systému. Pacienti, kteří byli nositeli ApoE4 alely měli menší tloušťku RNFL a prodlouženou latenci P1 v porovnání s pacienty bez ApoE4 alely. Přítomnost ApoE4 alely byla spojena s přítomností nekrotických a hemorrhagických lézí v mozku na MRI vyšetření odpovídajících následkům akutní intoxikace metanolem.

Závažnost acidemie u pacientů s akutní intoxikací metanolem je jedním z nejvýznamnějších parametrů ovlivňujících dynamiku změn latence P1, což nám umožnilo vytvořit model předpovídající dynamiku obnovení konduktivity zrakového nervu v průběhu čtyř let po akutní toxické optické neuropatii způsobené metanolem. Tento model adekvátně předpovídal výsledky měření u většiny pacientů ze sledovaného souboru. Nicméně, výsledky naší studie ukazují, že amplituda evokovaného potenciálu u pacientů přeživších otravu metanolem se nezvyšuje v letech následujících akutní neuronální poškození. Charakter asociace mezi amplitudou N1P1 a latencí P1, kdy asociace se postupně v průběhu čtyř let sledování stává méně významnou, odráží proces obnovení konduktivity v důsledku remyelinizace axonů zrakového nervu bez odpovídajícího obnovení amplitudy evokovaného potenciálu.

Další pokles amplitudy v důsledku chronické axonální degenerace byl zaznamenán prakticky u poloviny pacientů s měřitelným evokovaným potenciálem ve sledovaném období. Průměrný pokles amplitudy ve skupině 17 pacientů byl signifikantně vyšší než v celém souboru s maximální rychlostí poklesu o 5 až 7 mcV v průběhu 4 let. Abnormální amplituda N1P1 byla měřena na 24–33 % očí v rámci třetího vyšetření, což odpovídalo podílu pacientů s chronickou degenerací axonů sítnice zjištěnou měřením tloušťky RNFL v průběhu čtyř let. Zajímavé je, že signifikantní pokles amplitudy N1P1 byl zaznamenán u pacientů s původně normálním nálezem při prvním vyšetření po propuštění z nemocnice. Nezaznamenali jsme souvislost mezi dynamikou funkčních změn zrakového systému a opakovaně měřenými koncentracemi glukózy, TSH, vitamínů B_{12} a B_1. Avšak obnovení konduktivity bylo pomalejší u pacientů s vyšší koncentrací jaterní GGT, tedy u pacientů s chronickým abúzem alkoholu.

Apolipoprotein E není přímo zapojen do oxidace a eliminace metanolu, ale hraje významnou úlohu v rozvoji a metabolismu oka. Polymorfismus genů ApoE, pleiotropního proteinu ovlivňujícího riziko Alzheimerové choroby a kardiovaskulárních onemocnění, může hrát významnou roli jako genetická determinanta charakteru postižení zrakových funkcí u pacientů s toxickou optickou neuropatií v důsledku akutní otravy metanolem.

Zjistili jsme asociaci mezi polymorfismem ApoE a charakterem chronických morfologických a funkčních změn zrakového systému u pacientů přeživších otravu metanolem. Přítomnost ApoE4 alely může souviset jak s neuronálním, tak i s vaskulárním poškozením oční sítnice. V experimentálních studiích byl genotyp ApoE4 spojen s vaskulární patologií sítnice, sníženou koncentrací vaskulárního endoteliálního růstového faktoru (VEGF) a signifikantním poklesem hustoty synapsí v sítnici myších embryí (Maharshak et al., 2016). Hustota synapsí ve vrstvě neuronů sítnice u myší – nositelů ApoE4 byla signifikantně nižší než u nositelů

ApoE3 alely, což bylo doprovázeno nižším počtem presynaptických glutamatergických transportérů v neuronech sítnice ApoE4 nositelů (Antes et al., 2013).

Naše výsledky dokazují, že přítomnost alely ApoE4 může být spojena s chronickou neurodegenerací oční sítnice a progredující ztrátou funkcí zrakového nervu u pacientů přeživších akutní otravu metanolem. Dále, významná asociace mezi polymorfismem ApoE, axonální degenerací neuronů sítnice a MRI nálezem nekrotických a hemorrhagických lézí v mozku pacientů přeživších otravu metanolem poukazuje na možný patofyziologický vztah mezi dynamikou dlouhodobých zrakových následků a charakterem patologických změn v mozku v letech následujících po otravě. Naše studie upozorňuje na to, že chronické morfologické a funkční změny zrakového systému mohou souviset s genetickými rizikovými faktory pro chronická neurodegenerativní onemocnění v populaci pacientů přeživších akutní intoxikaci metanolem.

5.4. Prevence dlouhodobých zrakových následků akutních intoxikací metanolem (Publikace VI, VII)

Prevence dlouhodobých zrakových následků akutní toxické optické neuropatie způsobené metanolem musí být zahájena co nejdřív po expozici metanolu. Základem prevence je blokáda oxidace metanolu na toxické metabolity formaldehyd a kyselinu mravenčí. Právě akumulace kyseliny mravenčí v organismu má za následek inhibici mitochondriální cytochrom c oxidázy, buněčnou hypoxii, pokles utilizace kyslíku a syntézy ATP, zvýšenou produkci laktátu a metabolickou acidózu. Klíčová je včasná inhibice ADH buď etanolem nebo fomepizolem. Dostupnost etanolu v běžném životě umožňuje jeho využití jako vhodného antidota pro přednemocniční „první pomoc“ v případech podezření na akutní intoxikaci metanolem při hromadných otravách. Ve své studii jsme se zaměřili na vliv přednemocniční administrace etanolu v době české hromadné otravy metanolem v roce 2012 na prevalenci dlouhodobých zrakových následků u pacientů přeživších otravu.

Do studie bylo zařazeno 100 pacientů hospitalizovaných s akutní intoxikací metanolem ve věku 54 let (medián, IQR 38-61 let), 79 mužů a 21 žen, se známou koncentrací etanolu v krevním séru při příjmu do nemocnice a přednemocniční anamnézou zaměřenou na aplikaci etanolu v rámci první pomoci z důvodu podezření na akutní otravu. Třicet pacientů dostalo etanol v rámci přednemocniční péče od lékařů nebo zdravotnického nelékařského personálu, dalších dvanáct pacientů si samo aplikovalo etanol jako první pomoc před příjmem do nemocnice. Zbylých 58 pacientů etanol před příjmem do nemocnice nedostalo.

Demografická a laboratorní data pacientů jsou uvedena v Tabulce 14. Závažnost metabolické acidózy při příjmu do nemocnice, charakterizována pH arteriální krve, pCO_2, HCO_{3-}, deficitem bází, aniontovým oknem a koncentrací

laktátu, u pacientů přeživších otravu bez následků, s dlouhodobými následky a zemřelých je uvedena v tabulce 15. Etanol v krevním séru byl detekovatelný při příjmu u 42 pacientů. Klinické příznaky pacientů při příjmu do nemocnice zahrnovaly zhoršení zraku, gastrointestinální potíže, dušnost, bolesti na hrudníku a kóma v nejzávažnějších případech (viz Tabulka 16). Mezi dalšími příznaky otravy byly přítomny únava, bolest hlavy, točení hlavy, somnolence, tremor, křeče, srdeční zástava a zástava dýchání. Koncentrace etanolu v krevním séru při příjmu u asymptomatických pacientů byla vyšší než u pacientů s klinickými příznaky (10.9 mmol/L [IQR 1.1 – 29.8 mmol/L] versus 0 mmol/L [IQR 0 – 5 mmol/L]; p=0.014). Detailnější informace o nemocničních terapeutických opatřeních je uvedena v tabulce 17. Výsledek léčby pacientů je uveden v tabulce 18. Signifikantní pozitivní korelace byla přítomna mezi přednemocniční aplikací etanolu a vstupní koncentrací etanolu v krevním séru pacientů ($r = 0.713$, $p < 0.001$). Dále, signifikantní korelace byla přítomna mezi vstupní koncentrací etanolu v krevním séru pacientů a:

a) přežitím bez následků versus přežití s následky ze strany zraku/CNS nebo úmrtím ($r = 0.711$, $p < 0 .001$);

b) přežitím bez následků versus přežití s následky ze strany zraku/CNS versus úmrtí ($r = 0.693$, $p < 0.001$).

V rámci univariántní analýzy byly koncentrace etanolu v séru při příjmu do nemocnice i přednemocniční aplikace etanolu proměnnými významnými pro přežití pacientů bez následků z hlediska zraku / CNS (viz Tabulka 19).

V rámci bivariántní analýzy kombinace obou proměnných, tedy "koncentrace etanolu v séru při příjmu do nemocnice" a "přednemocniční aplikace etanolu jako první pomoci", s proměnnou "pH arteriální krve při příjmu do nemocnice" vysvětlovala 55.4 % a 48.9 % disperze ve výsledku léčby pacientů hospitalizovaných s akutní otravou metanolem (Tabulka 20).

Tabulka 14. Demografická a laboratorní data hospitalizovaných pacientů rozdělených dle skupin na základě výsledku léčby v rámci studie účinku přednemocniční aplikace etanolu jako antidota (medián, IQR).

		Věk [roky]	Čas [hodiny]	Metanol [mmol/L]	Etanol [mmol/L]	Mravenčan [mmol/L]	Osmolalní okno, [mmol/kg]	Glukóza [mmol/L]
	EtOH+	55	25	18.6	18.3	6.9	47	6.2
	(n=30)	47-64	17-48	9.1-43.1	7.1-28.1	1.2-13.0	21-73	5.7-7.6
	EtOH-	52	48	29.3	0.0	14.7	45.4	8.3
	(n=70)	37-60	24-48	13.0-56.3	0.0-0.0	11.7-16.7	23-77	6.2-13
Skupina I	**EtOH+**	54	26	15.6	19.3	4.9	36	6.0
(n=49)	**(n=27)**	47-62	14-48	9.2-41.5	9.3-29.8	1.0-11.3	22-73	5.7-7.2
	EtOH-	52	24	21.4	1.6	13.2	26	6.6
	(n=22)	35-58	22-48	12.3-31.8	0.0-25.0	7.9-15.3	19-44	6.1-8.2
Skupina	**EtOH+**	65	24	30.9	5.0	13.5	52	7.7
II	**(n=3)**	56-69	21-36	18.1-69.8	3.6-11.2	13.5-13.5	33-86	7.1-10.2
(n=30)	**EtOH-**	48	48	50.6	0.0	15.4	64	7.6
	(n=27)	37-58	30-50	25.0-82.1	0.0-0.0	13.5-18.5	39-100	6.0-11.3
Skupina	**EtOH+**	-	-	-	-	-	-	-
III	**(n=0)**							
(n=21)	**EtOH-**	58	48	34.1	0.0	15.5	65	12.7
	(n=21)	45-63	38-52	21.6-59	0.0-0.0	12.8-16.0	45-136	10.3-16.1
Celkem		54	41	28.7	0.0	14.4	46.8	7.3
(n=100)		38-61	24-48	12.3-54.9	0.0-12.7	8.9-16.6	21.7-75.9	6.0-11.2
$P_{(EtOH+ \text{ vs. } EtOH-)}$		0.185	0.090	0.068	<0.001***	0.005**	0.202	<0.001***
$P_{I(EtOH+ \text{ vs.}}$		0.212	0.636	0.459	0.883	0.093	0.151	0.220

EtOH-)							
$P_{II(EtOH+ vs. EtOH-)}$	0.070	0.266	0.643	0.250	-	0.706	0.848
P_{I-II}	0.581	0.030*	0.004**	<0.001***	0.044*	0.013*	0.041*
P_{I-III}	0.255	0.005**	0.080	<0.001***	0.202	0.015*	<0.001***
P_{II-III}	0.138	0.351	0.204	<0.001***	0.924	0.541	0.015*

Poznámky: EtOH+ – pacienti s přednemocniční administrací etanolu lékaři nebo zdravotnickým nelékařským personálem; EtOH- - pacienti bez přednemocniční administrace etanolu lékaři nebo zdravotnickým nelékařským personálem; Skupina I – přežití bez následků, Skupina II – přežití s následky, Skupina III - úmrtí. $P_{I,}$ $P_{II,}$ P_{III} – výsledky t-testu rozdílů mezi skupinami (*$\alpha \leq 0.05$; ** $\alpha \leq 0.01$; ***- $\alpha \leq 0.001$ (α- úroveň významnosti)).

Tabulka 15. Závažnost metabolické acidózy při příjmu do nemocnice u pacientů rozdělených na skupiny dle výsledku léčby v rámci studie účinku přednemocniční aplikace etanolu jako antidota (medián, IQR).

		pH	pCO_2, [kPa]	HCO_3^-, [mmol/L]	Deficit bází, [mmol/L]	Aniontové okno, [mmol/L]	Laktát [mmol/L]
	EtOH+	7.34	4.5	18.4	-6.1	20.3	2.5
	(n=30)	7.20-7.42	3.5-4.8	11.6-22.6	-1.5 - -14.6	18.3-28.6	1.9-3.6
	EtOH-	7.03	4.0	6.8	-23.2	32.3	6.0
	(n=70)	6.79-7.26	2.7-4.7	4.1-13.5	-11.3 - -29	22.3-39.8	1.9-9.3
Skupina I	**EtOH+**	7.36	4.6	20.9	-3.6	20.0	2.5
(n=49)	**(n=27)**	7.25-7.42	3.9-4.9	12.8-22.8	-1.2--12.8	18.1-26.8	1.9-3.4
	EtOH-	7.31	4.3	18.5	-4.5	23.2	2.1
	(n=22)	7.25-7.41	3.6-5.0	8.8-22.7	-1.7--15.6	18.2-28.5	1.7-4.0
Skupina	**EtOH+**	7.16	2.6	5.9	-22.1	30.9	4.8
II	**(n=3)**	7.01-7.18	2.3-3.3	4.7-8.7	-19.6--26.1	29.8-31.9	3.2-6.3
(n=30)	**EtOH-**	7.02	2.9	5.1	-25.4	32.7	3.2
	(n=27)	6.83-7.17	1.9-3.6	3.6-9.3	-19.1--27.5	25.3-37.7	1.4-7.4
Skupina	**EtOH+**	-	-	-	-	-	-
III	**(n=0)**						
(n=21)	**EtOH-**	6.79	4.5	5.2	-29	40.4	9.4
	(n=21)	6.65-6.93	3.5-6.1	3.9-7.7	-26.9--31.9	34.8-45.1	6.7-12.9
Celkem		7.18	4.1	8.8	-17.8	28.3	3.6
(n=100)		6.89-7.34	2.8-4.8	4.7-19.5	-3.7--27.7	19.4-36.3	1.9-7.8
$P_{(EtOH+ \text{ vs. } EtOH-)}$		<0.001***	0.587	<0.001***	<0.001***	<0.001***	<0.001***

$P_{I(EtOH+ \text{ vs. } EtOH-)}$	0.373	0.759	0.300	0.905	0.418	0.449
$P_{II(EtOH+ \text{ vs. } EtOH-)}$	0.601	0.976	0.939	0.961	0.666	0.991
P_{I-II}	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	0.111
P_{I-III}	<0.001***	0.181	<0.001***	<0.001***	<0.001***	<0.001***
P_{II-III}	<0.001***	0.041*	0.401	0.012*	0.042*	<0.001***

Poznámky: EtOH+ – pacienti s přednemocniční administrací etanolu lékaři nebo zdravotnickým nelékařským personálem; EtOH- - pacienti bez přednemocniční administrace etanolu lékaři nebo zdravotnickým nelékařským personálem; Skupina I – přežití bez následků, Skupina II – přežití s následky, Skupina III - úmrtí. P_I, P_{II}, P_{III} – výsledky t-testu rozdílů mezi skupinami (*$\alpha \leq 0.05$; ** $\alpha \leq 0.01$; ***- $\alpha \leq 0.001$ (α- úroveň významnosti)).

Tabulka 16. Klinické příznaky při příjmu do nemocnice u pacientů hospitalizovaných s otravou metanolem rozdělených na skupiny na základě výsledku léčby v rámci studie účinku přednemocniční aplikace etanolu jako antidota.

		Asymptomatičtí pacienti, n (%)	VD n (%)	GI n (%)	D n (%)	CP n (%)	RA n (%)	C n (%)
Skupina I (n=49)	EtOH+ (n=27)	16 (59%)	4 (15%)	7 (26%)	1 (4%)	1 (4%)	0 (0%)	0 (0%)
	EtOH- (n=22)	6 (27%)	10 (45%)	18 (82%)	10 (45%)	1 (5%)	0 (0%)	4 (18%)
Skupina II (n=30)	EtOH+ (n=3)	1 (33%)	3 (100%)	2 (67%)	1 (33%)	0 (0%)	0 (0%)	1 (33%)
	EtOH- (n=27)	0 (0%)	8 (30%)	8 (30%)	8 (30%)	1 (4%)	0 (0%)	8 (30%)
Skupina III (n=21)	EtOH- (n=21)	0 (0%)	12 (57%)	10 (48%)	11 (53%)	7 (33%)	3 (14%)	15 (71%)
Celkem (n=100)	EtOH+ (n=30)	17 (57%)	7 (23%)	9 (30%)	2 (7%)	1 (3%)	0 (0%)	1 (3%)
	EtOH- (n=70)	6 (9%)	30 (43%)	36 (51%)	29 (41%)	9 (13%)	3 (4%)	27 (39%)
$P_{tot(EtOH+ vs. EtOH-)}$	p	<0.001***	0.064	0.048	0.001***	0.146	0.250	<0.001***
	OR (C.I.)	13.95 (4.6-42.1)	0.41 (0.15-1.07)	0.41 (0.16-1.01)	0.10 (0.02-0.46)	0.23 (0.03-1.93)	0.000 (--)	0.06 (0.01-0.43)
$P_{I(EtOH+ vs. EtOH-)}$	p	0.025	0.018*	<0.001***	<0.001***	0.88	1.000	0.021
	OR (C.I.)	3.88 (1.15-13.04)	0.21 (0.05-0.81)	0.08 (0.02-0.31)	0.05 (0.01-0.40)	0.81 (0.05-13.70)	0.000 (--)	0.000 (---)
$P_{II(EtOH+ vs. EtOH-)}$	p	0.002	0.016*	0.197	0.894	0.735	1.000	0.894
	OR (C.I.)	0.000 (---)	0.000 (---)	4.75 (0.38-60.15)	1.19 (0.09-15.04)	0.000 (---)	0.000 (--)	1.19 (0.09-15.04)

Poznámky: EtOH+ – pacienti s přednemocniční administrací etanolu lékaři nebo zdravotnickým nelékařským personálem; EtOH- - pacienti bez přednemocniční administrace etanolu lékaři nebo zdravotnickým nelékařským personálem; Skupina I – přežití bez následků, Skupina II – přežití s následky, Skupina III - úmrtí. VD – porucha zraku, GI – gastrointestinální potíže, D – dušnost, CP – bolest na hrudníku, C – kóma, RA – zástava dýchání. Chi2-test (*$\alpha \leq 0.05$; ** $\alpha \leq 0.01$; ***- $\alpha \leq 0.001$ (α- úroveň významnosti).

Tabulka 17. Léčba poskytnutá pacientům v nemocnici v rámci studie účinku přednemocniční aplikace etanolu jako antidota (dle skupin na základě výsledku léčby).

		Alkalinizace	Etanol	Fomepizol	Foláty	CVVHD/ CVVHDF	IHD
Skupina I (n=49)	EtOH+ (n=27)	8 (30%)	21 (78%)	6 (22%)	20 (74%)	10 (37%)	8 (30%)
	EtOH- (n=22)	12 (55%)	19 (86%)	2 (9%)	19 (86%)	7 (32%)	9 (41%)
Skupina II (n=30)	EtOH+ (n=3)	2 (67%)	2 (67%)	2 (67%)	2 (67%)	1 (33%)	1 (33%)
	EtOH- (n=27)	25 (93%)	18 (67%)	8 (30%)	22 (81%)	13 (48%)	12 (44%)
Skupina III (n=21)		20 (95%)	16 (76%)	7 (33%)	13 (62%)	15 (71%)	5 (24%)
Celkem (n=100)	EtOH+ (n=30)	10 (33%)	23 (77%)	8 (27%)	22 (73%)	11 (37%)	9 (30%)
	EtOH- (n=70)	57 (81%)	53 (76%)	17 (24%)	54 (77%)	35 (50%)	26 (37%)
$P_{tot\ (EtOH+\ vs.\ EtOH-))}$	p	<0.001***	0.919	0.801	0.683	0.220	0.493
	OR (C.I.)	0.11 (0.04-0.30)	1.05 (0.39-2.89)	1.13 (0.43-3.01)	0.82 (0.31-2.18)	0.58 (0.24-1.39)	0.73 (0.29-1.82)
$P_{I\ (EtOH+\ vs.\ EtOH-)}$	p	0.078	0.440	0.216	0.288	0.703	0.409
	OR (C.I.)	0.35 (0.11-1.14)	0.55 (0.12-2.52)	2.86 (0.5-15.85)	0.45 (0.10-2.00)	1.26 (0.38-4.14)	0.61(0.19-1.99)
$P_{II\ (EtOH+\ vs.\ EtOH-))}$	P	0.156	1.000	0.197	0.543	0.626	0.713
	OR (C.I.)	0.16 (0.01-2.63)	1.00 (0.08-12.56)	4.75 (0.4-60.15)	0.46 (0.03-6.06)	0.54 (0.04-6.67)	0.63 (0.05-7.75)

Poznámky: EtOH+ – pacienti s přednemocniční administrací etanolu lékaři nebo zdravotnickým nelékařským personálem; EtOH- - pacienti bez přednemocniční administrace etanolu lékaři nebo zdravotnickým nelékařským personálem; Skupina I – přežití bez následků, Skupina II – přežití s následky, Skupina III - úmrtí. IHD – intermitentní hemodialýza; CVVHD/CVVHDF – kontinuální hemodialýza/hemodiafiltrace.

Tabulka 18. Přednemocniční administrace etanolu versus výsledek léčby akutní otravy metanolem (n=100).

	Skupina I	Skupina II	Skupina III
Přednemocniční administrace etanolu lékaři/zdravotníky - **ano** (n=30)	27 (90.0%)	3 (10.0%)	0 (0.0%)
Přednemocniční administrace etanolu lékaři/zdravotníky - **ne** (n=70)	22 (31.4%)	27 (38.6%)	21 (30.0%)
p	<0.001***	0.004**	<0.001***
OR (C.I.)	19.64 (5.38-71.7)	0.2 (0.05-0.64)	0.0 (-)
Přednemocniční administrace etanolu včetně laické (n=42)	38 (90.5%)	4 (9.5%)	0 (0.0%)
Přednemocniční etanol nebyl podán (n=58)	11 (19.0%)	26 (44.8%)	21 (36.2%)
p	<0.001***	<0.001***	<0.001***
OR (C.I.)	40.6 (12.0-137.7)	0.1 (0.04-0.41)	0.0 (-)

Poznámky: Skupina I – přežití bez následků, Skupina II – přežití s následky, Skupina III - úmrtí. Chi^2-test (*$\alpha \leq 0.05$; ** $\alpha \leq 0.01$; ***$\alpha \leq 0.001$ (α-úroveň významnosti).

Tabulka 19. Parametry signifikantní pro přežití bez zrakových / CNS následků otravy metanolem v rámci studie účinku přednemocniční aplikace etanolu jako antidota (univariántní analýza).

	Intercept	β	SE	OR	LE 95% CI	UE 95% CI	p	Cox & Snell R^2	Nagelkerke R^2	Hosmer-Lemeshow R^2
S-EtOH	-6.319	2.576	0.504	**13.139**	4.892	35.291	0.000	0.439	0.586	0.417
pH	-4.013	2.253	0.411	9.515	4.249	21.308	0.000	0.405	0.540	0.375
HCO_3	-2.839	1.527	0.284	4.602	2.637	8.033	0.000	0.321	0.427	0.279
„První pomoc“	-2.197	2.977	0.661	**19.636**	5.378	71.703	0.000	0.273	0.365	0.230
GCS	-2.086	1.556	0.441	4.742	1.997	11.258	0.000	0.141	0.189	0.110
MetOH	-1.841	0.941	0.279	2.563	1.485	4.425	0.001	0.122	0.162	0.094
Čas	-1.917	1.068	0.352	2.910	1.459	5.804	0.002	0.115	0.153	0.088
Laktát	-1.396	0.933	0.332	2.541	1.325	4.873	0.005	0.105	0.142	0.082

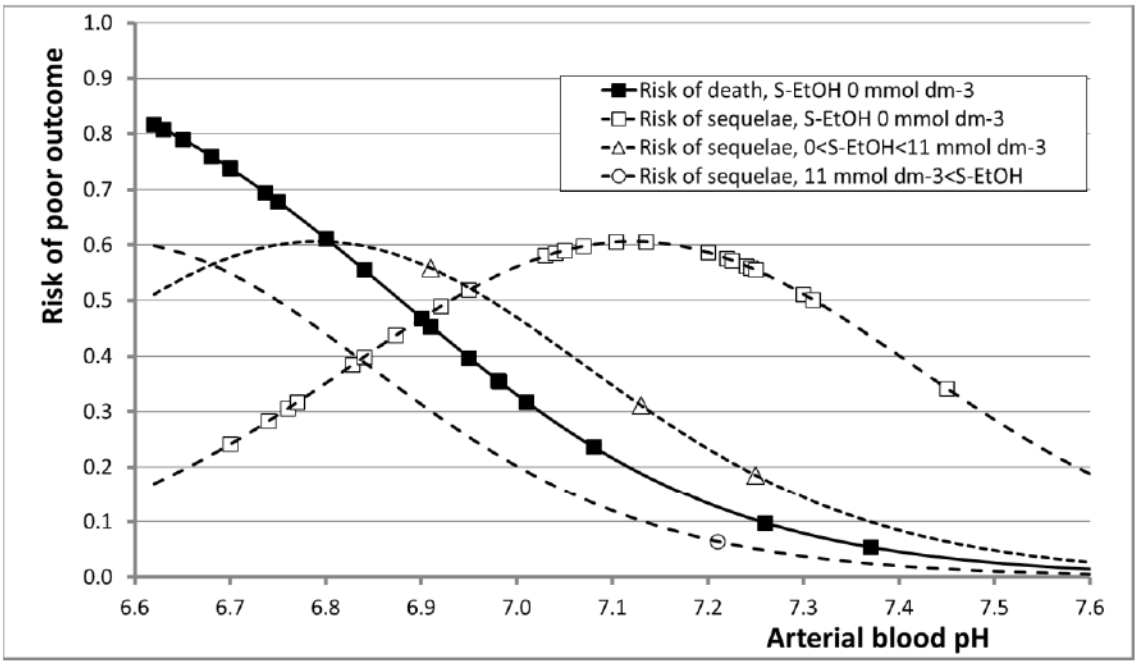
Poznámky: OR – odds ratio; LE 95%CI – dolní hranice 95 % konfidenčního intervalu; UE 95%CI – horní hranice 95 % konfidenčního intervalu; "První pomoc" – přednemocniční administrace etanolu; pH – pH arteriální krve; GCS – Glasgow coma scale; MetOH – koncentrace metanolu; Laktát – koncentrace laktátu; Čas – délka časového intervalu od požití metanolu do začátku hospitalizace.

Tabulka 20. Bivariántní regresní analýza proměnných významných pro přežití bez následků pacientů s akutní otravou metanolem v rámci studie účinku přednemocniční aplikace etanolu jako antidota.

		Intercept	β_1	SE_1	p_1	β_2	SE_2	p_2	Adjusted OR (Exp β_1)	LE 95% CI	UE 95% CI	Hosmer-Lemeshow R^2
pH	První pomoc	-5.894	2.166	0.456	0.000	2.725	0.809	0.001	**8.726**	**3.567**	**21.344**	0.489
HCO_3	První pomoc	-4.090	1.258	0.304	0.000	2.308	0.720	0.001	3.519	1.941	6.380	0.373
GCS	První pomoc	-4.101	2.868	0.685	0.000	1.458	0.523	0.005	17.595	4.593	67.404	0.299
MetOH	První pomoc	-4.139	2.974	0.698	0.000	0.972	0.333	0.004	19.562	4.978	76.877	0.296
Laktát	První pomoc	-3.914	3.434	0.824	0.000	0.913	0.411	0.027	30.989	6.158	155.943	0.349
Čas	První pomoc	-4.027	2.857	0.709	0.000	1.075	0.412	0.009	17.415	4.342	69.843	0.288
pH	EtOH	-8.393	2.092	0.533	0.000	1.817	0.478	0.000	**8.103**	**2.852**	**23.018**	0.554
HCO_3	EtOH	-7.188	2.137	0.518	0.000	1.018	0.339	0.003	8.476	3.070	23.404	0.485
GCS	EtOH	-7.824	2.417	0.490	0.000	1.435	0.641	0.025	11.216	4.289	29.328	0.462
MetOH	EtOH	-8.130	2.554	0.534	0.000	0.933	0.378	0.014	12.863	4.514	36.660	0.461

Poznámky: OR – odds ratio; LE 95%CI – dolní hranice 95 % konfidenčního intervalu; UE 95%CI – horní hranice 95 % konfidenčního intervalu; "První pomoc" – přednemocniční administrace etanolu; pH – pH arteriální krve; GCS – Glasgow coma scale; MetOH – koncentrace metanolu; Laktát – koncentrace laktátu; Čas – délka časového intervalu od požití metanolu do začátku hospitalizace.

Pacienti s pozitivní koncentrací etanolu v krevním séru při příjmu do nemocnice měli OR pro přežití bez následků versus nepříznivý výsledek léčby (úmrtí nebo přežití s poškozením zraku / mozku) 8.10 (2.85 – 23.02 CI 95%; $p < 0.001$) po adjustaci na závažnost acidemie (pH arteriální krve). Dále, pacienti, kteří dostali etanol v rámci přednemocniční péče od zdravotnického personálu, měli OR pro přežití bez následků versus nepříznivý výsledek léčby (úmrtí nebo přežití s poškozením zraku / mozku) 8.73 (3.57 – 21.34 CI 95%; $p < 0.001$) po adjustaci na závažnost acidemie. Výsledky logistické regrese pravděpodobnosti úmrtí nebo přežití s poškozením zraku a mozku v závislosti na vstupní koncentraci etanolu v séru a pH arteriální krve jsou uvedeny na obrázku 14.



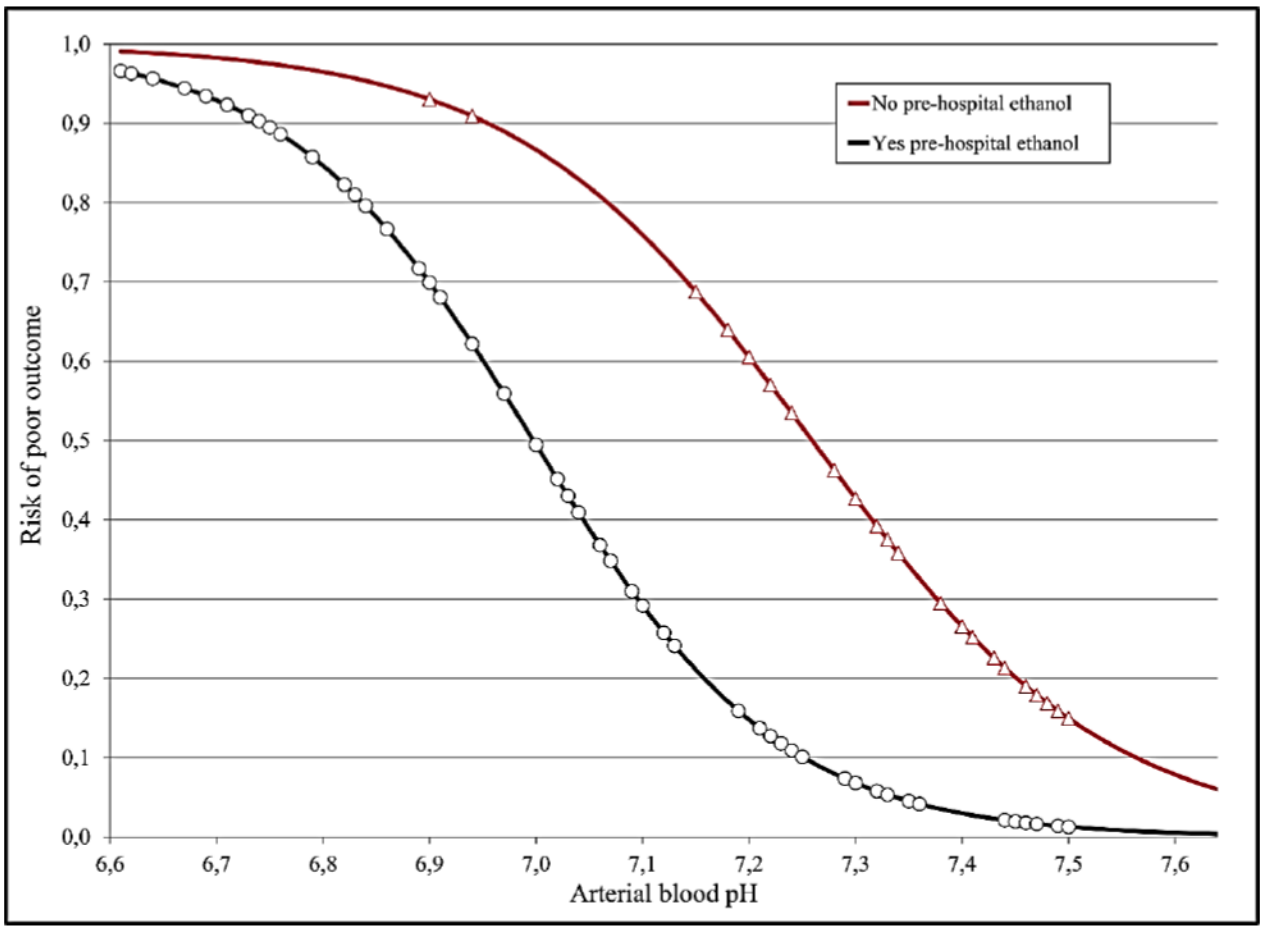
Obrázek 14. Riziko úmrtí a poškození zraku a mozku v závislosti na koncentraci etanolu v séru a pH arteriální krve při příjmu do nemocnice. Celkem n=100: úmrtí (n=21) + přežití s následky otravy (n=30) + přežití bez následků (n= 49). S-EtOH – koncentrace etanolu v krevním séru při příjmu do nemocnice.

Pravděpodobnost úmrtí klesala exponenciálně se zvýšením pH arteriální krve. Všichni zemřelí pacienti měli nedetekovatelný etanol v krevním séru při příjmu do nemocnice. Avšak pravděpodobnost poškození zraku a mozku u pacientů přeživších otravu závisela nejen na stupni acidemie, ale také na koncentraci etanolu v séru při příjmu do nemocnice, se signifikantním posunem vrcholu křivky doleva. Tato skutečnost znamená, že vyšší koncentrace etanolu lépe chránila pacienty se stejnou pH arteriální krve proti poškození zraku a mozku. Například u pacientů s pH 7.0 pravděpodobnost přežití s následky byla 59 % (nedetekovatelný etanol v séru) *versus* 41% (koncentrace etanolu < 11 mmol/L) *versus* 16% (koncentrace etanolu > 11 mmol/L).

Výsledek logistické regrese pravděpodobnosti nepříznivého výsledku léčby (úmrtí nebo poškození zraku a mozku) u pacientů, kteří dostali etanol od zdravotnických pracovníků v rámci přednemocniční péče v porovnání s pacienty, kterým antidotum nebylo poskytnuto, je uveden na obrázku 15. Pravděpodobnost nepříznivého výsledku klesala exponenciálně se zvýšením pH arteriální krve, ale rychlost poklesu byla vyšší u pacientů, kteří dostali přednemocniční etanol, tedy tito pacienti měli menší riziko nepříznivého výsledku léčby při stejném pH arteriální krve.

Nepříznivý výsledek léčby akutní otravy metanolem je především důsledkem pozdního odhalení pacientů a s tím spojené opožděné diagnózy a pozdějšího zahájení léčby antidotem, etanolem nebo fomepizolem (Megarbane et al., 2001, 2005, 2010, 2014; Zakharov et al., 2014, 2015). Závažnost metabolické acidózy při příjmu do nemocnice, jako jeden z prognostických faktorů, závisí mimo jiné na délce časového úseku mezi požitím metanolu a zahájením léčby antidotem (Mahieu et al., 1989; Paasma et al., 2012). Včasná korekce acidemie a rychlá eliminace kyseliny mravenčí hemodialýzou jsou také klíčovými faktory pro úspěšnou léčbu a

prevenci poškození zraku a mozku u pacientů s akutní otravou metanolem (Zakharov et al, 2014; Vaneckova et al., 2015).



Obrázek 15. Riziko nepříznivého výsledku léčby (úmrtí nebo poškození zraku a mozku) v závislosti na poskytnutí etanolu zdravotnickým personálem v rámci přednemocniční péče.

Pacienti s pozitivním etanolem v krevním séru měli mírnější acidemii při příjmu do nemocnice, vyšší pH arteriální krve a koncentraci bikarbonátu, nižší deficit bází, aniontové okno, koncentraci laktátu a kyseliny mravenčí při srovnatelně stejné době uplynulé od ingesce metanolu do příjmu jako u pacientů, kterým přednemocniční etanol nebyl poskytnut. Tato skutečnost ukazuje, že ADH byla dostatečně blokována množstvím etanolu poskytnutého v rámci první pomoci.

Přitom pacienti byli schopni adekvátně hyperventilovat, což znamená, že podání etanolu jako první pomoci u pacientů s podezřením na otravu neovlivnilo jeden z důležitých mechanismů regulace pH arteriální krve a kompenzace acidemie (McMartin et al., 1980; Hovda et al., 2005c; 2008; Zakharov et al., 2015).

Závěrem naší studie bylo doporučení podat etanol v rámci přednemocniční péče pacientům s podezřením na akutní otravu metanolem k prevenci poškození zraku a mozku při hromadných otravách metanolem, které bylo publikováno v časopise *Annals of Emergency Medicine*. Protokol podávání přednemocničního etanolu obsahoval doporučenou dávku 1.8-2.0 ml/kg váhy 40% roztoku etanolu všem dospělým pacientům s podezřením na otravu s výjimkou pacientů ve stavu bezvědomí. Cílem tohoto opatření bylo dosažení koncentrace etanolu v séru 22 mmol/L (1000 mg/L) a inhibice ADH co nejdřív po ingesci metanolu, tedy dříve než diagnóza mohla být potvrzena výsledkem toxikologické analýzy. Toto doporučení mělo důležitý preventivní dopad zejména v případech, kdy cesta do nemocnice disponující dialyzačním zařízením a toxikologickou laboratoří mohla trvat delší dobu nebo se vyskytly jiné faktory, které mohly vést ke zpoždění diagnózy a zahájení léčby.

6. ZÁVĚR

V naší studii jsme prezentovali data získaná v průběhu české hromadné otravy metanolem v roce 2012 a v rámci prospektivní longitudinální kohortové studie zrakových následků u pacientů přeživších otravu, která proběhla v letech 2013 – 2017 ve Všeobecné fakultní nemocnici v Praze.

Hlavní výsledky a závěry studie mohou být shrnuty v následujících bodech:

6.1. Prevalence a charakter zrakových následků akutní otravy metanolem:

6.1.1. Prevalence zrakových následků akutní toxické optické neuropatie v souboru pacientů přeživších otravu dosahuje 40 % a může být podhodnocena v případě absence komplexního oftalmologického a neurooftalmologického vyšetření u každého pacienta po propuštění z nemocnice. Nepřítomnost subjektivních zrakových potíží nevylučuje abnormální morfologický nález na oční sítnici, zejména pokles tloušťky vrstvy nervových vláken, jakož i abnormální funkce zrakového nervu, zejména poruchu konduktivity a snížení amplitudy evokovaného potenciálu.

6.1.2. Abnormální tloušťka vrstvy nervových vláken sítnice byla zjištěna na OCT RNFL u 38 % pacientů ze sledovaného souboru při prvním vyšetření za 4.9 ± 0.6 měsíců po propouštění z nemocnice. Prodloužení latence vlny P1 evokovaného potenciálu způsobené demyelinizací axonů zrakového nervu bylo zaznamenáno na 43 % pravých a 50 % levých očí, abnormální nízká nebo neměřitelná amplituda N1P1 evokovaného potenciálu způsobená akutní ztrátou axonů zrakového nervu byla zaznamenána na 24 % očí pacientů přeživších otravu metanolem.

6.1.3. Abnormální morfologický nález na oční sítnici a dysfunkce zrakového nervu byly doprovázeny poklesem ostrosti zraku u 32 % pacientů, poruchou barvocitu u 34 % pacientů, výpadky zorného pole a zúžením perimetru u 44 %

pacientů a snížením kontrastní citlivosti u 56 % pacientů ze sledovaného souboru při prvním vyšetření.

6.2. Dynamika chronických morfologických změn oční sítnice v průběhu čtyř let sledování:

6.2.1. Akutní poškození gangliových buněk neuronů oční sítnice kyselinou mravenčí při otravě metanolem bylo následováno chronickou neurodegenerací a progredující ztrátou axonů přibližně u 25 % pacientů ze sledovaného souboru. Tento proces byl doprovázen další progredující ztrátou zrakových funkcí u většiny z těchto pacientů. Většina pacientů s abnormální tloušťkou RNFL měla také známky poškození mozku na MRI.

6.2.2. Rychlost poklesu tloušťky RNFL v důsledku ztráty neuronálních axonů u pacientů přeživších těžkou otravu metanolem se závažnou acidemií byla signifikantně vyšší než rychlost fyziologického poklesu spojeného se stárnutím. Vstupní pH arteriální krve pacientů při příjmu do nemocnice, jako ukazatel závažnosti metabolické acidózy, má prognostický význam pro dynamiku chronické ztráty axonů zrakového nervu v letech následujících po otravě.

6.2.3. Přednemocniční aplikace etanolu jako antidota a vyšší rychlost eliminace a korekce acidemie za intermitentní hemodialýzy souvisely s větší tloušťkou RNFL u pacientů ze sledovaného souboru. Vliv druhu antidota použitého v nemocnici (etanol versus fomepizol) a substituce foláty na dynamiku chronických změn oční sítnice byl nesignifikantní.

6.3. Klinické, biochemické a genetické determinanty chronických změn zrakových funkcí v průběhu čtyř let po akutní optické neuropatii:

6.3.1. Demyelinizace axonů zrakového nervu v důsledku akutního toxického účinku kyseliny mravenčí má reverzibilní charakter. Obnovení konduktivity zrakového nervu po akutním poškození myelinového obalu nastalo u více než 80 % pacientů ze sledovaného souboru v průběhu 4 let v důsledku remyelinizace axonů zrakového nervu, přičemž nejvyšší rychlost tohoto procesu byla zaznamenána v průběhu prvních dvou let po propouštění z nemocnice.

6.3.2. Délka časového intervalu od požití metanolu do začátku hospitalizace a zahájení léčby antidotem, závažnost metabolické acidózy (pH arteriální krve, deficit bází, aniontové okno při příjmu do nemocnice) a věk pacienta byly nejvýznamnějšími nezávislými proměnnými, které měly vliv na dynamiku remyelinizace a obnovení konduktivity zrakového nervu.

6.3.3 Amplituda evokovaného zrakového potenciálu, abnormální přibližně u každého čtvrtého pacienta ze sledovaného souboru při propuštění z nemocnice, vykazovala tendenci k dalšímu poklesu. U poloviny pacientů s vybavitelným evokovaným potenciálem byl zaznamenán pokles amplitudy N1P1 o 1.0 mcV a více v období sledování.

6.3.4. Pacienti, kteří byli nositelé ApoE4 alely, měli menší tloušťku RNFL a prodlouženou latenci P1 evokovaného potenciálu v porovnání s pacienty bez ApoE4 alely. Přítomnost ApoE4 alely byla spojena s přítomností nekrotických a hemorrhagických lézí v mozku na MRI vyšetření odpovídajících následkům akutní intoxikace metanolem.

6.4. Prevence dlouhodobých zrakových následků akutních intoxikací metanolem:

6.4.1. Prevence poškození zraku v důsledku akutní toxické optické neuropatie způsobené metanolem musí být zahájena co nejdřív po expozici metanolu. Klíčová je včasná inhibice alkoholdehydrogenázy. Podání etanolu v rámci přednemocniční péče pacientům s podezřením na akutní otravu metanolem má důležitý preventivní dopad a chrání před poškozením zraku při hromadných otravách metanolem.

6.4.2. Základem prevence poškození zraku u pacientů s podezřením na otravu metanolem je dosažení koncentrace etanolu v séru 22 mmol/L (1000 mg/L) a inhibice ADH co nejdřív po ingesci metanolu, tedy dříve než diagnóza bude potvrzena výsledkem toxikologické analýzy. Toto opatření má preventivní dopad zejména v případech, kdy cesta do nemocnice disponující dialyzačním zařízením a toxikologickou laboratoří může trvat delší dobu nebo se vyskytnou jiné faktory, které mohou vést k opožděnému stanovení diagnózy a zahájení léčby.

6.4.3. Druh antidota použitého v rámci nemocniční léčby (etanol versus fomepizol) k inhibici ADH a substituce foláty neměly význam pro výskyt a charakter zrakových následků u pacientů ze sledovaného souboru. Rychlejší korekce acidemie a eliminace kyseliny mravenčí a metanolu z krevního séra za intermitentní hemodialýzy v porovnání s kontinuálními modalitami může mít pozitivní vliv na výsledek léčby a přežití pacientů bez poškození zraku.

6.5. Praktická doporučení na základě prospektivní studie zrakových následků akutních otrav metanolem:

6.5.1. Všichni pacienti, kteří přežili akutní intoxikaci metanolem, byměli absolvovat kompletní oftalmologické vyšetření při propuštění z nemocnice

zahrnující vyšetřen ostrosti zraku, kontrastní citlivosti, perimetru, barvocitu a disku zrakového nervu (fundoskopii) k posouzení charakteru zrakových následků otravy.

6.5.2. Po uplynutí dvou měsíců od propuštění z nemocnice, nezbytných pro odeznění symptomů pseudopapillitis a otoku oční sítnice, všichni pacienti, kteří přežili akutní intoxikaci metanolem, by měli absolvovat druhé oftalmologické vyšetření zahrnující kromě vyšetření uvedených v bodu 6.5.1. také optickou koherenční tomografii s měřením tloušťky vrstvy nervových vláken sítnice k posouzení morfologického stavu oční sítnice a vyšetření zrakových evokovaných potenciálů k posouzení funkce zrakového nervu. Toto vyšetření umožní včas odhalit dlouhodobé zrakové následky otravy zejména u pacientů bez subjektivních potíží.

6.5.3. Abnormální výsledky měření VEP a / nebo OCT RNFL u pacientů přeživších akutní otravu metanolem svědčí o vysoké pravděpodobnosti neurologických následků, zejména o přítomnosti nekrotických a hemorrhagických ložisek v bazálních gangliích a subkortikální bílé hmotě. Pacienti s abnormálním VEP / OCT nálezem by měli absolvovat MRI mozku a neurologické vyšetření.

6.5.4. Vysoká prevalence zrakových následků akutních otrav metanolem a negativní dynamika chronických neurodegenerativních změn oční sítnice a zrakového nervu u pacientů přeživších otravu v letech následujících po otravě svědčí o nezbytnosti dispenzarizace a pravidelných kontrolních vyšetření očním lékařem zaměřených na včasné odhalení těchto změn a terapeutickou intervenci vedoucí ke zlepšení kvality života pacientů. Doporučení o dispenzarizaci může být zařazeno do národních standardů a doporučených postupů pro léčbu akutních intoxikací metanolem.

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8. SEZNAM TABULEK

Tabulka 15. Závažnost metabolické acidózy při příjmu do nemocnice u pacientů rozdělených na skupiny dle výsledku léčby v rámci studie účinku přednemocniční aplikace etanolu jako antidota (medián, IQR).

Tabulka 16. Klinické příznaky při příjmu do nemocnice u pacientů hospitalizovaných s otravou metanolem rozdělených na skupiny na základě výsledku léčby v rámci studie účinku přednemocniční aplikace etanolu jako antidota.

Tabulka 17. Léčba poskytnutá pacientům v nemocnici v rámci studie účinku přednemocniční aplikace etanolu jako antidota (dle skupin na základě výsledku léčby).

Tabulka 18. Přednemocniční administrace etanolu versus výsledek léčby akutní otravy metanolem (n=100).

Tabulka 19. Parametry signifikantní pro přežití bez zrakových / CNS následků otravy metanolem v rámci studie účinku přednemocniční aplikace etanolu jako antidota (univariántní analýza).

Tabulka 20. Bivariántní regresní analýza proměnných významných pro přežití bez následků pacientů s akutní otravou metanolem v rámci studie účinku přednemocniční aplikace etanolu jako antidota.

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11. PŘÍLOHY: KOPIE HLAVNÍCH ORIGINÁLNÍCH PUBLIKACÍ

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ORIGINAL ARTICLE

Long-term visual damage after acute methanol poisonings: Longitudinal cross-sectional study in 50 patients

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Context. Visual disturbances due to the toxic effect of formic acid in acute methanol poisonings are generally transient. The subjective symptoms of visual toxicity may resolve within few weeks and fundoscopic signs of acute optic neuropathy subside within 1–2 months; therefore, the prevalence of long-term visual sequelae in the population of survivors of poisonings may be underestimated. *Objective.* To study the prevalence and character of long-term visual sequelae of acute methanol poisonings based on the data from the Czech mass methanol outbreak in 2012. *Patients and methods.* A total of 50 patients with confirmed methanol poisoning were included in this longitudinal cross-sectional study, median age: 48 (range, 23–73) years. The following tests were performed: optical coherence tomography or OCT with evaluation of the retinal nerve fibers layer (RNFL), visual evoked potentials (VEP), magnetic resonance imaging (MRI) of brain, complete ocular examination (visual acuity/field, color vision, contrast sensitivity, and fundus), neurological examinations, and biochemical tests. *Results.* Of 50 patients, 7/50 (14%) were discharged with diagnosed visual sequelae and 6/50 (12%) were discharged with both visual and central nervous system sequelae of poisoning. On the follow-up examination, 20/50 (40%) of the patients had long-term visual sequelae, with 8% of blindness. A total of 38% of the patients had abnormal (28% borderline) findings on RNFL, and 40% had abnormal (18% borderline) VEP. Among the patients discharged without detected visual sequelae, 8/37 (22%) had abnormal RNFL and VEP. Patients with visual sequelae had brain lesions more often (70% vs. 27%, $p < 0.01$). MRI identified optic nerve lesions in 2/20 cases with abnormal VEP only. The groups with and without visual sequelae differed in serum methanol, ethanol, HCO_{3-}, formate, pH, anion gap, and base deficit (all $p < 0.01$). Visual disturbances on admission and coma were more prevalent in the patients with visual sequelae ($p < 0.05$). Patients with positive serum ethanol on admission were 93% less likely to have optical axonal damage (OR: 0.07 (95% CI: 0.01–0.8); $p < 0.05$). No association was found between visual sequelae and type of antidote administered, mode of hemodialysis, or

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folate substitution. Pre-hospital administration of ethanol seemed beneficial: these patients were 90% less likely to have abnormal RNFL findings (OR: 0.10 (95% CI: 0.02–0.52); $p < 0.01$). *Conclusions.* The long-term visual sequelae were clearly underestimated on discharge, suggesting a significantly higher amount of patients with long-term sequelae than earlier reported. Thorough examinations before discharge and during follow-up will likely uncover a higher morbidity also after methanol poisonings in general.

Keywords: acute methanol poisoning; hospital treatment; treatment outcome; long-term visual damage; health sequelae of poisoning

Introduction

Acute methanol poisoning is a medical emergency where rapid blocking of alcohol dehydrogenase is important because of the toxic effect of its metabolite, formic acid, on the retina and optic nerve (see the review on the topic of formic-acid-induced ocular toxicity in Barceloux DG et al (2002)).[1] Formic acid/the formate anions have a strong cytotoxic effect due to inhibition of mitochondrial cytochrome c oxidase activity causing cell hypoxia.[2,3] The retinal ganglion cells and their axons, which form the optic nerve, are selectively vulnerable to histotoxic hypoxia, likely because of their high energy dependence.[4–6]

In many cases, a complete recovery with resolution of changes of the fundus, improvement of visual acuity, and extinction of subjective signs and symptoms will occur. However, the symptoms of long-term visual damage may persist in 10–30% of patients.[7–9] These symptoms may include peripheral constriction of visual fields and/or central scotoma, with reduced visual acuity, loss of color vision, and finally, blindness in the most serious cases.[10–12] The results of a follow-up study by Paasma et al in Estonia suggest the possibility of new—or unrecognized—visual complications in up to 36% of patients during the six years following the acute poisoning.[13]

Nevertheless, longitudinal cross-sectional studies of the prevalence of long-term visual sequelae of acute methanol poisonings with objective and subjective ophthalmologic examinations performed several months after discharge are absent. The symptoms of pseudopapillitis may persist for several weeks after acute intoxication and subside completely after 1–2 months following discharge from the hospital.[14–17] Therefore, the actual morphology of long-term changes of retinal nerve fibers layer (RNFL) and the functional state of the visual pathway can be estimated with reliability not earlier than two months after discharge and resolving of residual symptoms of toxic optic neuropathy.

Further, the problem of evaluating the prevalence and the character of long-term visual sequelae of acute methanol poisoning is that the objective methods of optical coherence tomography (OCT), magnetic resonance imaging (MRI) of brain, and visual evoked potentials (VEP) are not routinely performed during the hospitalization of poisoned patients.

In this study we report the data based on the recent methanol mass poisoning in the Czech Republic in 2012[18] specifically addressing the prevalence and character of long-term visual damage (visual sequelae of acute methanol poisoning persisting more than two months after discharge from hospitals when the acute pseudopapillitis resolved and the edema of optic nerve and retina completely subsided) in survivors.

Materials and methods

Patients and procedures

The study was designed as a longitudinal cross-sectional examiner-masked study. During the Czech methanol outbreak from September 3, 2012 until January 1, 2013, all cases of confirmed methanol poisonings treated in hospitals were documented using a standardized admission protocol developed during the Norwegian methanol outbreak in 2002–2004,[7] and discharge reports of all hospitalized patients with confirmed diagnosis and results of neurological and ophthalmologic examinations on admission, during hospitalization, and on discharge were collected and analyzed in the Czech Toxicological Information Centre.

On admission, the laboratory investigations included serum concentrations of methanol, ethanol, formate, lactate, electrolytes, and bicarbonate, arterial blood gases, anion and osmolal gaps, glucose, urea, creatinine, liver enzymes, complete blood count, hematocrit, and serum proteins. The diagnosis was established if (1) a history of recent ingestion of illicit spirit was available, and serum methanol concentration was more than 6.2 mmol/L (20 mg/dL), or (2) there was a history/clinical suspicion of methanol poisoning, serum methanol detectable, and at least two of the following were present: pH less than 7.3, serum bicarbonate less 20 mmol/L (20mEq/L), and anion gap more than 19 mmol/L (19mEq/L). The ophthalmologic examinations during hospitalization and on discharge included fundus examination and standard ophthalmic tests.

All the patients with confirmed acute methanol poisonings who survived poisoning and were discharged from hospitals were included in the study. The patients with diabetes mellitus, arterial hypertension, cataract, amblyopia, and sarcoidosis were included in the study to prevent the selection bias (exclusion of elderly and possibly more vulnerable subjects from the study population). The patients who did not admit participation on the follow-up clinical examination and did not sign the informed consent were excluded from the longitudinal study.

The patients were treated in accordance to the American Academy of Clinical Toxicology and European Association of Poisons Centres and Clinical Toxicologists (AACT/EAPCCT) practice guidelines for the treatment of methanol poisoning.[1] Bicarbonate was given as a buffer to the patients with metabolic acidosis. Ethanol or fomepizole were used as antidotes to block alcohol dehydrogenase enzyme. Intermittent hemodialysis (IHD) was performed in 30 patients, and continuous venovenous hemodialysis/hemodiafiltration (CVVHD/HDF) was performed in 45 patients. Folates were administered in 63 patients: folic acid (Acidum folicum Léčiva, tbl. 10 mg, Zentiva, Czech Republic) in 35, and

folinic acid (Calcium folinate Hospira, amp. 20 ml, 10 mg/ml Hospira UK Limited, Great Britain) in 28 subjects. Corticosteroids were not administered to the patients with visual disturbances.

Investigation protocol

The clinical examination protocol 3–8 months after discharge from hospitals included complete ocular examination and standard ophthalmic tests, OCT with RNFL thickness evaluation, VEP, MRI of the head, neurological and neuropsychological examinations, biochemical tests (electrolytes, glucose, glycohemoglobin, albumin, pre-albumin, urea, creatinine, bilirubin, liver enzymes, cholesterol, lipids, thyroid-stimulating hormone (TSH), vitamin B_{12}, carbohydrate-deficient transferrin (CDT), complete blood count, and hematocrit), ethyl glucuronide in urine, physical examination, arterial blood pressure measurement, and standardized questionnaire forms. The examiners were masked to the serum methanol and formic acid concentrations on admission, severity of poisoning, clinical course, treatment measures and outcomes (results of ocular examination, neurological examination, and CT of brain) in methanol-poisoned patients on discharge from hospitals, as well as to each other's results.

RNFL thickness was measured on the OCT SPECTRALIS Tracking Laser Tomography (Heidelberg Engineering GmbH, Heidelberg, Germany, software version 5.8.3) and compared with the normative database. On the diagram, the green area represented the mean RNFL thickness of normal eyes. Normal eyes were defined as those that fell within 5–95 percentile of normal distribution. Measured values in this range were considered "within normal limits." The red area represented values below the 1 st percentile of RNFL thickness of normal distribution. Measured values at this range were considered "abnormal—outside normal limits." The yellow area represented values between the 1 and 5 percentile of normal distribution. Measured values in this range were considered "borderline," and did not indicate that measured values related to abnormal state.

VEP examination was performed on two-channel device TruTrace 4 Alien Technik CZ. Monocular checkerboard pattern-reversal stimulation was used, with frequency of 1.5 c/s, angular size of the monitor $6^0 \times 5^0$ from the fixation point, and angular size of checkerboard squares of 40'. Luminance of the white and black squares was 84 cd/m2 and 57 cd/m2, respectively. Bandwidth of the amplifier was 1 Hz–1 kHz, the evoked response was registered from the Oz-Fz derivation. At each eye, the examination was performed twice in order to check reproducibility of the evoked complex. We evaluated latencies of waves N1, P1, and N2, and amplitudes N1P1 and P1N2. The measured values in patients were compared with our laboratory reference values determined as the central 95% interquantile interval of data measured on a group of 30 healthy individuals. Four criteria of abnormality were chosen: (1) missing evoked response, (2) wave P1 latency $>$ 117 ms, (3) interocular difference of waves P1 latencies $>$ 6 ms, and (4) amplitude of evoked complex $<$ 3 μV. The result was categorized as abnormal if at least one of the above-mentioned four criteria was fulfilled.

The best-corrected visual acuity (BCVA) was established on standard Snellen charts at a distance of 6 meters (20 feet). The visual acuity was considered pathologic if worse than 6/60. The visual field was examined by means of static perimetry (Medmont perimeter M700 automated perimeter; Medmont International Pty Ltd, Vermont, Australia, Neurological test, threshold strategy). The findings were considered pathologic if there were any defects in the visual field. The color vision was examined by means of Lanthony's 15-D test (Richmond Products Albuquerque NM, U.S.A.). The finding was considered normal up to 3 crossings, borderline with 3–7 crossings and pathologic with more than 7 crossings. The contrast sensitivity was examined by means of Pelli–Robson contrast sensitivity test (Clement Clarke International Ltd, Essex, UK). The finding was considered normal (1.35 and better), borderline (1.20–1.05), or pathologic (worse than 1.05). The fundus (posterior pole of the eye) was examined by means of biomicroscopy on the slit lamp with the + 78 diopters lens (Volk-lens type). The finding was considered pathologic, if any related pathology of the optic nerve head and/or the adjacent retina was present).

The patients underwent MRI on Gyroscan Philips 1.5 T system with the following protocol: axial T2-weighted image with slice thickness (THK) 6.0/0.6 mm through the whole brain, with parameters: repetition time (TR) 4241 ms, time to echo (TE) 100 ms, flip angle (FA) 90°, fluid-attenuated inversion recovery or FLAIR: TR 11000 ms, TE 140 ms, inversion time (TI) 2800 ms, FA 90°, T1-weighted image: TR 569 ms, TE 15 ms, FA 69°, T2-weighted image fast field echo: TR 665 ms, TE 23 ms, FA 18°, single-shot diffusion-weighted image: TR 2901 ms, TE 75 ms, FA 90°, T1-weighted after administration of Gd-DTPA and in coronal images centered to the orbital region T2-weighted image with suppression of fat (SPIR): TR 5506 ms, TE 100 ms, FA 90°.

The patients were further divided in two groups according to the results of clinical examination: Group I, the patients without findings compatible with long-term visual sequelae of acute methanol poisonings; and Group II, the patients with long-term visual sequelae of acute methanol poisonings.

The patients were considered having long-term visual sequelae of acute methanol poisonings if

1. abnormal RNFL with abnormal VEP were present in at least one eye, or
2. abnormal RNFL with borderline VEP or abnormal VEP with borderline RNFL were present in at least one eye with concurrent pathologic findings on fundus, perimeter, color vision, and contrast sensitivity, and
3. possible comorbidities, like diabetes mellitus, arterial hypertension, sarcoidosis, cataract, amblyopia, and glaucoma were considered and excluded as a cause of abnormal RNFL and VEP findings, and there was a causal relationship of abnormal findings with confirmed acute methanol poisoning and toxic optic neuropathy diagnosed in hospitals.

The rationale for inclusion of the "borderline" results of measurements was to detect the patients with abnormal functional parameters of optic nerve conductivity suggesting myelin sheaths damage and axonal loss with possible retrograde degeneration of retinal nerve ganglion cells during the following months (borderline RNFL thickness within 1–5 percentile of normal distribution might indicate the initial stage of this process) and the patients with abnormal RNFL and borderline conductivity changes of optic nerve suggesting possible remyelination after acute myelin damage.

Statistical analyses

The laboratory and clinical data were compared using Two-Sample Assuming Unequal Variances (Equal Means), Two-Sample F-Test for Variances, bias test, and two-sample Kolmogorov–Smirnov test. The data were expressed as medians with range. Chi-square tests were used to examine the differences between treatment modalities (type of antidote, mode of hemodialysis, and folate therapy), laboratory parameters (pH, bicarbonate, anion gap, serum methanol, and lactate), and visual sequelae. Statistically significant treatment modalities and laboratory parameters were subsequently used in bivariate logistic regression as independent variables. Variables that showed significant association with the visual outcome on bivariate analysis were included in a multiple linear regression model with the visual outcome as the dependent variable and the tested laboratory investigations and treatment measures as independent variables. All statistical calculations were carried out on level of significance $\alpha = 0.05$. Statistical analysis was performed using Excel (Microsoft, USA), and the formal calculations were produced in QC Expert software 3.1 (Trilobyte, Pardubice, Czech Republic) and in IBM SPSS ver. 17.0 and Statistica SF ver. 10.0 (both licensed to 1 st Faculty of Medicine of Charles University in Prague).

Ethics

The study was approved by the General University Hospital Ethics Committee in Prague, Czech Republic. Informed consent was obtained from the participants of the study. The study adhered to the tenets of the Declaration of Helsinki.

Results

Demographic characteristics

A total of 121 cases of methanol poisonings occurred during the period from September 3, 2012 until January 1, 2013 (see Fig. 1). One hundred and one patients were treated in 30 hospitals in 11 regions of the Czech Republic, of whom 21 patients died in hospitals. Further, 20 persons died at home or before hospital admission, giving a total mortality of 41 patients (34%). According to the results of ocular and neurological examinations and CT of brain on discharge from hospitals, there were 60/80 (75%) survivors without, and 20/80 (25%) with sequelae: visual impairment was found within the standard ophthalmologic examination on discharge (visual acuity, perimeter, color vision, and fundoscopy) in nine (six patients with visual acuity loss, eight cases of perimeter and color vision defects, and nine cases with residual fundus lesions), central nervous system (CNS) impairment was found within the standard neurological examination, CT of brain (was provided during hospitalization in eight patients) and MRI of brain (was provided during hospitalization in two patients) in four cases (basal ganglia lesions on CT in three cases, severe motor impairment in two cases), and both visual and CNS involvements (four cases of blindness; three patients with visual acuity loss, perimeter and color vision defects; and seven patients with residual fundus lesions) in seven cases.

All 80 patients who survived the acute intoxication were invited to participate in a one-day outpatient clinical

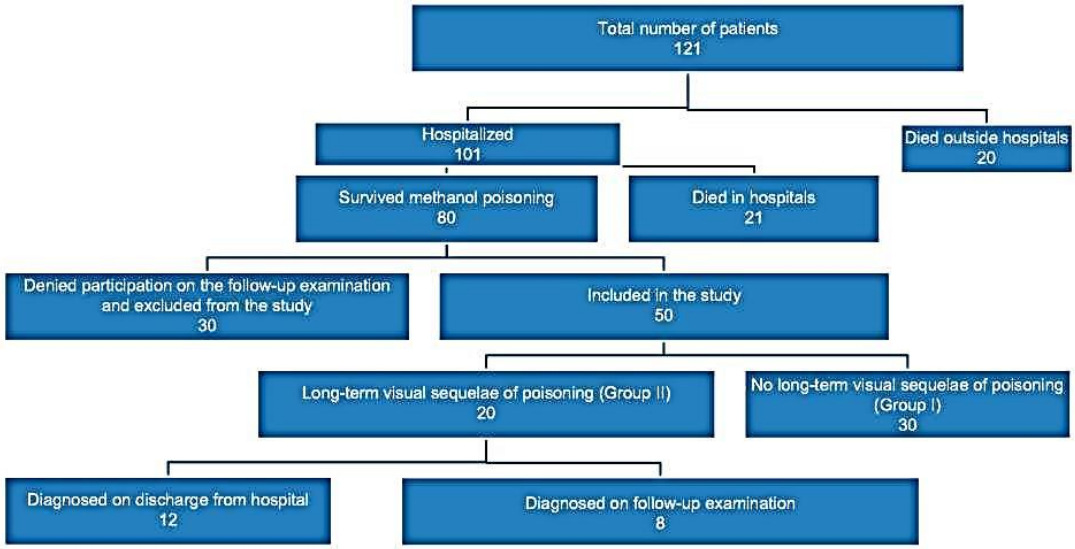

Fig. 1. Flowchart of the study design: The outbreak of methanol poisonings in the Czech Republic from 3.9.12 to 1.1.13.

examination. Fifty patients (63%) with median age of 48 (range: 23–73) years, 41 males and 9 females, agreed and were included in the study and absolved the follow-up clinical examinations 3–8 months after the acute poisoning. Of them, 13/50 (26%) patients were discharged from hospitals with diagnosed sequelae of poisoning: seven patients with visual sequelae, and six with visual and CNS sequelae, whereas 37/50 (74%) were discharged without health sequelae from the methanol poisoning.

For the patients included in the study, the median ingested volume of toxic spirits was 0.3 (range: 0.1–1.5) L containing approximately 50% methanol and 50% ethanol in different kinds of strong alcoholic beverages with a total alcohol content around 40% alcohol by volume (ABV, v/v). In 19 cases, ethanol was administered per os as a "first aid antidote" by the ambulance medical/paramedic staff before admission to the hospital.

Only 26% of these patients were diagnosed within 12 hours after the methanol ingestion, 60% within 48 hours, and 8% later than 48 hours. A medical history of chronic alcohol abuse was present in 64% of the cases. To control the effect of chronic alcohol abuse on the results of the study, the clinical examination protocol included measurements of CDT, gamma-glutamyltransferase (GGT), serum vitamin B_{12}, and urine screening of ethyl glucuronide on the day of examination. Regarding somatic diseases, five patients had diabetes mellitus type II, one patient had sarcoidosis, and 19 patients had arterial hypertension. Furthermore, two patients had cataract in one eye, and two patients had amblyopia. To estimate possible effect of comorbidities on the results of examinations, the clinical investigation protocol included the key laboratory parameters, instrumental examinations (e.g., glycohemoglobin and glycemia, ocular fundoscopy and electromyography in diabetes; arterial blood pressure, cholesterol, lipids, fundoscopy in arterial hypertension, and so on), and the study of the history of diseases from medical documentation.

Results of longitudinal clinical examination 3–8 months after discharge

A variety of visual sequelae of acute methanol poisoning were found in 20 (40%) of the patients (Group II), with four cases (8%) of complete blindness (Table 1). There was a positive correlation between abnormal RNFL and abnormal VEP ($p = 0.001$). Further, the associations were present between both abnormal RNFL and VEP and color vision defects, diminished contrast sensitivity, visual field defects, and fundus lesions (all $p < 0.001$). Most of the patients in the group with long-term visual sequelae had color vision and visual field defects, diminished contrast sensitivity, and fundus lesions. Three patients in this group had diabetes mellitus type II, two of them became completely blind during the first day of hospitalization with acute poisoning, having fundus lesion as typically seen in methanol poisonings. The third one had bilateral central and peripheral visual field defects in addition to abnormal RNFL and VEP findings, reduced color vision, typical fundus lesions, and bilateral symmetrical lesions in putamen on MRI of brain. These three patients in Group II had normal glycohemoglobin on the day of examination suggesting adequate therapeutic control of glycemia. Ten patients in this group had arterial hypertension sufficiently controlled with ACE inhibitors (normal arterial blood pressure on the day of examination, corresponding data from the medical documentation), and one patient had sarcoidosis without the signs of intraocular sarcoidosis. Therefore, in all these patients the possibility of competing causal relationship was examined and excluded. Finally, thirteen patients had a history of chronic alcoholism with positive ethyl glucuronide test in urine in eight cases and high serum CDT level in five cases.

The objective findings in Group I (no sequelae) were isolated (e.g., abnormal latency of evoked complex with normal thickness of RNFL) and did not comply with the criteria described previously, or the causal relationship with methanol poisoning was not proved. As an example of the patients from "No sequelae" group we provide the data on the single patient with abnormal RNFL included in Group I: 48-year-old male with medical history of chronic alcohol abuse and confirmed methanol poisoning was examined 4 months after discharge and had borderline latencies of N1 peaks, normal latencies of P1 and N2 peaks, and normal amplitudes of evoked complex on both eyes, normal RNFL in right eye and abnormal thinning of RNFL only in the low temporal segment in left eye with normal other segments, normal visual fields and contrast sensitivity on both eyes, hereditary

Table 1. Findings on ophthalmologic examinations and MRI of the brain in the patients with acute methanol poisoning 3–8 months after discharge from hospitals.

Groups	RNFL abnormal	RNFL borderline	VEP abnormal	VEP borderline	Visual fields abnormal	Color vision abnormal	Contrast sensitivity abnormal	Fundus lesion	Visual acuity abnormal	MRI brain damage
Group I ($n = 30$)	1 (3%)	12 (40%)	4 (13%)	7 (23%)	8 (27%)	3 (10%)	11 (37%)	2 (7%)	3 (10%)	**8 (27%)**
Group II ($n = 20$)	18 (90%)	2 (10%)	16 (80%)	2 (10%)	14 (70%)	14 (70%)	17 (85%)	12 (60%)	13 (65%)	**14 (70%)**
Total ($n = 50$)	19 (38%)	14 (28%)	20 (40%)	9 (18%)	22 (44%)	17 (34%)	28 (56%)	14 (28%)	16 (32%)	**22 (44%)**
$P_{I=II}$	<0.001*	<0.001*	<0.001*	0.229*	0.003*	<0.001*	<0.001*	<0.001*	<0.001*	**0.003***

Group I – without long-term visual sequelae; Group II – with long-term visual sequelae; RNFL – retinal nerve fibers layer, VEP – visual evoked potential, MRI – magnetic resonance imaging. Visual acuity abnormal - if the best corrected visual acuity established on standard Snellen charts at 6 meters (20 feet) distance worse than 6/60. MRI brain damage - symmetrical necrosis of basal ganglia (putamen, globus pallidum) and other brain lesions (brainstem, nucleus caudatum, cerebellum, deposits in white matter, and optical nerve atrophy) compatible with the diagnosis of acute methanol poisoning. $P_{I=II}$ – results of Chi^2 test of the difference between Group I and Group II.
*-significant value.

anomalous trichromacy (protanomaly), and physiological findings on fundoscopy. This patient had high serum methanol on admission (2640 mg/L), but presented to hospital with protective serum level of ethanol (1310 mg/L) due to co-ingestion of different alcoholic drinks during at least the last two days, without metabolic acidosis (pH: 7.44; bicarbonate 23.7 mmol/L, and anion gap 11 mmol/L). The patient was asymptomatic on admission with clinical features of inebriation only; he was treated with ethanol, CVVHD, and calcium folinate, and was discharge from hospital without sequelae of poisoning. The comorbidities included arterial hypertension on ACE, hypercholesterolemia, and alcoholic hepatopathy. Analysis of these data led us to the conclusion about the absence of long-term visual sequelae of poisoning despite isolated abnormal RNFL finding.

Only 12/20 (60%) of the patients with determined long-term visual sequelae were discharged from hospitals with recorded pathologic findings, ranging from a pseudopapillitis in regression to optic atrophy with complete blindness. One patient with residual fundus lesion (regressing acute pseudopapillitis) and normal visual acuity, color vision, and perimeter on discharge from hospital had normal results of subjective tests and VEP and RNFL measurements on the follow-up examination and was classified as "survivor without long-term visual sequelae." Therefore, forty percent of the patients in this group were discharged from hospitals without detected visual damage caused by methanol poisoning.

Significantly more patients with long-term visual sequelae had pathologic findings on brain MRI compatible with sequelae of acute methanol poisoning as compared with the patients without visual sequelae (70% vs. 27%, $p < 0.01$). In the patients with visual sequelae, there was a symmetrical damage of putamen found significantly more often as compared with the patients without visual sequelae (58% vs. 10%, $p < 0.01$). Among the patients without visual sequelae, the signs of CNS damage were more polymorphic (globus pallidus in 13%, subcortical lesion in 3%, lemniscus medialis in 3%, nucleus dentatus in 3%, and thalamus in 3%). Morphologic changes of the optic nerve were identified on MRI only in 2/20 with abnormal VEP findings.

On the day of examination, positive urine ethyl glucuronide test was determined in 54% of the cases, and serum CDT level was elevated (>2%) in 30% of cases. These two tests of chronic alcoholism correlated significantly ($p < 0.01$) identifying a group of patients with likely chronic alcohol abuse during the period after the methanol poisoning. No significant difference was found in serum vitamin B_{12} level (450 ± 170 ng/ml vs. 368 ± 50 ng/ml), TSH (2.44 ± 0.55 mIU/L vs. 2.31 ± 0.37 mIU/L), serum glucose (5.07 ± 0.61 mmol/L vs. 5.61 ± 0.73 mmol/L), CDT ($3 \pm 2.2\%$ vs. $3 \pm 1.3\%$), and results of ethyl glucuronide test on the day of examination, between the groups with and without long-term visual sequelae.

Laboratory Data, Clinical Features, Treatment in Hospitals, and Visual Sequelae of Poisonings

Laboratory data and clinical features on admission are presented in Tables 2 and 3.

Five laboratory parameters, arterial blood pH, serum bicarbonate, lactate, methanol, and ethanol concentrations on admission, were used in univariate and multivariate logistic regression in order to predict the long-term disruption of axonal integrity of retinal nerve fibers in patients with acute methanol poisoning (Table 4).

The association between different treatment modalities given to the patients with acute methanol poisoning and long-term axonal damage of retinal nerve fibers was tested by univariate logistic regression. No association was found with type of antidote (ethanol or fomepizole) administered in hospitals, mode of hemodialysis (IHD or CVVHD/HDF), or folate substitution. The only significant association was found with pre-hospital ethanol administration: these patients were 90% less likely to have abnormal RNFL findings (OR = 0.10; 95% CI = 0.02–0.52; $p < 0.01$).

Discussion

We found the prevalence of long-term visual sequelae of acute methanol poisonings to be significantly higher than the prevalence of visual damage on discharge from hospitals and earlier stated in the literature, what can be ascribed to the application of more robust objective techniques of OCT and VEP measurements. At least 8/37 (22%) of the patients without subjective complaints of visual disturbances and being discharged from the hospital without detected visual damage had abnormal findings both on RNFL and VEP. Alto-

Table 2. Laboratory data on admission in the patients with acute methanol poisoning (medians with ranges).

Groups	Age (years)	pH	pCO_2 (kPa)	HCO_3^- (mmol/L)	BD (mmol/L)	AG (mmol/L)	S-Lactate (mmol/L)	S-MetOH (mmol/L)	S-EtOH (mmol/L)	S-Formate (mmol/L)	S-Glucose (mmol/L)
Group I ($n = 30$)	46	7.3	4.2	17.8	7.5	22.3	1.9	21.8	2.2	7.0	6.3
	23–69	7.0–7.5	1.5–6.1	2.5–23.7	0.1–27.2	11.1–41.9	0.7–17.1	2.7–93.6	0–96.8	0–31.1	4.4–11.5
Group II ($n = 20$)	48	7.1	3	6.4	22.8	32.6	2.0	52.6	0	15.5	6.6
	25–73	6.7–7.4	1.7–5.2	2.7–21.8	1.6–38.1	16.6–54.8	0.9–16.3	12.5–228.1	0–8.0	8.9–22.5	4.8–19.8
Total ($n = 50$)	48	7.2	3.8	11.4	16.1	24.5	1.9	28.7	0.1	11.7	6.4
	23–73	6.7–7.5	1.5–6.1	2.5–23.7	0.1–38.1	11.1–54.8	0.7–17.1	2.7–228.1	0–96.8	0–31.1	4.4–19.8
$P_{I=II}$	0.746	**0.001***	**0.086***	**0.007***	**0.001***	**0.006***	**0.099***	**0.008***	**0.007***	**0.003***	**0.078**

Group I – without long-term visual sequelae; Group II – with long-term visual sequelae; AG – anion gap, BD – base deficit, S-MetOH – serum methanol, S-EtOH – serum ethanol. $P_{I=II}$ – results of Chi^2 test of the difference between Group I and Group II.
*-significant value.

Table 3. Clinical features on admission in—and treatment given to—patients with acute methanol poisoning.

Groups	Sex (M/F)	Alcoholism	Dose of toxic spirit*, mL	Time to treatment*, hours	Visual disturbances	Coma (GCS < 8)	Antidote (Fo/E)	HD (IHD/ CVVHD)	Folates (Yes/No)	First aid ethanol (Yes/No)
Group I ($n = 30$)	25/5	19 (63%)	410 (100–1000)	24 (2–96)	7 (23%)	0	5/25 (17%/83%)	13/9 (43%/30%)	23/7 (77%/23%)	17/11 (57%/37%)
Group II ($n = 20$)	16/4	13 (65%)	510 (100–1500)	34 (3–96)	13 (65%)	9	5/11 (25%/55%)	9/8 (45%/40%)	14/6 (70%/30%)	2/18 (10%/90%)
Total ($n = 50$)	41/9	32 (64%)	300 (100–1500)	24 (2–96)	20 (40%)	9	10/36 (20%/72%)	22/17 (44%/34%)	37/13 (74%/26%)	19/29 (38%/58%)
$P_{I=II}$	0.963	**0.419**	**0.420**	**0.685**	**0.012***	**<0.001***	**0.073**	**0.672**	**0.838**	**<0.001***

Group I – without long-term visual sequelae; Group II – with long-term visual sequelae; M – male, F - female, GCS – Glasgow coma scale; Fo - fomepizole, E – ethanol, HD – hemodialysis, IHD – intermittent hemodialysis, CVVHD – continuous veno-venous hemodialysis. $P_{I=II}$ – results of Chi^2 test of the difference between Group I and Group II. * - medians with ranges.

Table 4. Univariate and multivariate logistic regression of laboratory parameters on admission significant for the long-term axonal damage of retinal nerve fibers in the patients with acute methanol poisoning.

	Unadjusted		Adjusted	
Parameter	OR	(95% CI)	OR	(95% CI)
S-EtOH	**0.10**	**(0.02–0.52)**	**0.07**	**(0.01–0.8)**
S-MetOH	**4.25**	**(1.52–11.94)**	**5.59**	**(1.49–21.03)**
pH	**2.41**	**(1.35–4.32)**	**3.92**	**(1.59–25.95)**
HCO_3^-	**2.35**	**(1.14–4.84)**	1.16	(0.01–2.49)
S-Lactate	1.82	(0.69–4.8)	1.43	(0.36–5.65)

Note: Bold text indicates statistically significant result at $p < 0.05$, OR - odds ratio, CI - confidence interval; S-EtOH – serum ethanol concentration on admission (two cut-offs: negative serum ethanol concentration *versus* positive serum ethanol concentration); S-MetOH – serum methanol concentration (three cut-offs: < 15 mmol/L, 15–25 mmol/L, and > 25 mmol/L); pH – arterial blood pH on admission (three cut-offs: < 7.14, 7.14–7.25, > 7.25); HCO_3^- - serum bicarbonate concentration (three cut-offs: < 9.3 mmol/L, 9.3–13.8 mmol/L, > 13.8 mmol/L); S-Lactate – serum lactate concentration (three cut-offs: < 1.2 mmol/L, 1.2–2.9 mmol/L, > 2.9 mmol/L). Unadjusted effect – results of univariate regression analysis (prediction of axonal damage based on one selected parameter with no other covariates). Adjusted effects – results of multivariate regression analysis (prediction of axonal damage based on the selected parameter in the presence of other covariates that affect the outcome).

gether, 40% of the examined patients had abnormal findings complying with the criteria of long-term visual damage due to acute methanol poisoning. Another 26% of the examined patients had abnormal or borderline findings on RNFL, whereas 22% of the patients had abnormal or borderline VEP, however, not complying with the criteria as stated in the "Materials and Methods" section.

Methanol poisoning has a high mortality and incidence of long-term sequelae in spite of complex and resource-consuming treatment.[1] There are reports describing visual lesions to be variable and either transient or permanent with a variety of possible dynamics.[10–13,19] There are two main reasons why the data on prevalence, character, and development of long-term visual sequelae of methanol poisonings are scarce: Firstly, precise objective examination methods such as OCT, VEP, and MRI, are not routinely performed during the hospitalization or on discharge in the patients poisoned with methanol, preventing the subclinical cases of retinal nerve fiber layer and optical nerve damage to be found. Secondly, only three follow-up clinical studies have been performed in patients more than two months after the acute poisoning, when the residual symptoms are thought to have subsided completely, but these studies were retrospective and did not have any of the above-mentioned objective tests available.[10,11,13]

The higher prevalence of visual sequelae of acute methanol poisonings compared with earlier studies,[7–9] as well as the high prevalence of subclinical findings without subjective signs of visual damage and "borderline" findings, underlines the necessity of comprehensive ocular examination of the patients on discharge from hospitals. This should include not only the standard ophthalmologic examination, but also the examination of axonal integrity of the retinal nerve fibers and the functional state of the optic nerve. In facilities without available OCT and MRI equipment, the patients should be examined by other available methods such as VEP, visual

field testing, color vision test, and contrast sensitivity test. If any of these results are abnormal or borderline and there is no other pathology to explain it, these patients should be referred to other facility to perform OCT and brain MRI examinations.

There was a significant correlation between RNFL and VEP findings, as well as positive association between long-term visual sequelae and pathologic MRI findings in the basal ganglia of the brain. This suggests that in medical facilities without available OCT and MRI equipment, functional examination of the optic nerve (VEP) can provide a good alternative screening to determine the target group of patients for further thorough examination including OCT and brain MRI examination.

Severity of metabolic acidosis, serum methanol, and ethanol concentrations on admission were the laboratory parameters correlating significantly with the long-term visual sequelae of acute methanol poisoning. Earlier studies have demonstrated the role of pH as a prognostic factor for permanent visual disturbances.[10,11,20] The patients with visual sequelae had twice as high concentration of formate in blood on admission to hospitals. Eells et al showed that formic acid accumulation was much higher in the rat eye as compared with the rat brain, possibly due to its slower oxidation in the eye.[21] They found that concentration of formic acid in the vitreous humor and in the retina was 50% higher than in the retrolaminar segment of the optic nerve and in the brain. Positive serum ethanol on admission likely plays a role as a protective factor by blocking alcohol dehydrogenase and preventing the toxic metabolite formation and accumulation.

Analysis of the clinical symptoms on admission in our patients suggested that the subjects with visual disturbances on admission (blurry or cloudy vision, central visual field defects, alterations in light, color, and depth perception, etc.) had long-term visual sequelae significantly more often than those without early subjective visual symptoms (65% vs. 23%, $p < 0.05$, Table 3). In the study of Sanaei-Zadeh H et al, early subjective symptoms of visual toxicity as blurred or snowfield vision recovered within up to a maximum of two weeks after discharge.[11] This fact corresponds with the data on the resolve of acute pseudopapillitis, when the edema of optic disk and peripapillary retinal edema typically completely subside within several weeks.[12,16,17] On the contrary, prolongation of optic nerve conductivity, decrease in amplitude of visual evoked complex, and abnormal thickness of RNFL registered 3–8 months after discharge, when the symptoms of acute toxic neuropathy were completely resolved, represented the long-term effects of optic nerve demyelination, axonal degeneration, and retrograde retinal ganglion cells death.[22,23] Finally, long-term visual sequelae were found in all nine patients who were admitted to hospitals in coma (GCS < 8), of whom four (44%) became completely blind. Coma on admission being associated with poor outcome is also found in other studies.[24,25]

No difference was found in the prevalence and character of long-term visual sequelae between the groups of patients treated with different antidotes (fomepizole vs. ethanol), folate substitution (folic/folinic acid "yes" vs. "no), or mode of hemodialysis (IHD vs. CVVHD/HDF), notwithstanding the rate of elimination of formic acid on IHD known to be 2.2 times higher than that on CVVHD/HDF.[26] However, the patients with pre-hospital ethanol had better visual outcomes than those without pre-hospital ethanol administration ($p < 0.01$).

Further prospective studies are necessary to determine the development of the abnormal findings, as well as the "borderline" changes; whether they regress, suggesting possible regeneration, or whether it could be an ongoing progress developing over time as suggested by Paasma et al.[13]

The prevalence of long-term visual sequelae after acute methanol poisonings was significantly higher than the prevalence of visual damage on discharge from hospitals and earlier stated in the literature. About every fourth patient without subjective signs of visual disturbances discharged from hospitals without detected visual sequelae had both morphological and functional abnormal findings of the retina and the optic nerve. Two-thirds of the patients who survived the methanol poisoning had abnormal or at least borderline RNFL and/or VEP findings 3–8 months after discharge, suggesting long-term damage to the retinal ganglion cells and their axons. Further progression—or regression—of the "borderline" changes is possible during the months/years following the poisoning.

Strengths and limitations

The study has some principal limitations. The lack of "baseline" OCT-RNFL, VEP, and MR examinations in the study population before acute methanol poisoning, during the treatment, and on discharge from hospitals prevents comparison of the results of morphological and functional tests from the follow-up examination with corresponding "baseline" parameters before methanol exposure and on discharge. Therefore, the possibility of abnormal VEP and/or RNFL findings before methanol poisonings in the patients with predisposing conditions (diabetes mellitus and others) cannot be excluded and the degree of possible worsening cannot be measured. Nevertheless, the study population was relatively young with median age of 48 years, the proportion of the patients with co-morbidities potentially affecting the results of measurements was relatively low, and the parameters studied within the follow-up examination (glycohemoglobin, arterial blood pressure, and others) allowed us to exclude other causes of abnormal visual findings. Further, even if available, the results of VEP examinations during the treatment and on discharge could not be directly compared with the results of follow-up examinations of optic nerve latency prolongation and neuronal loss due to conductivity block caused by myelin sheaths swelling in acute optic neuropathy.[27] Finally, reliable measurement of RNFL is possible only when the peripapillary retinal edema completely subsides, to prevent the false negative results with "normal" RNFL thickness.[27]

The study was not controlled for the time to presentation (time between methanol ingestion and start of treatment), time

of blood collection for laboratory analysis (the laboratory parameters measured from the first blood sample collected in a hospital were assumed as the parameters measured on admission), and the treatment modalities (choice of antidote and mode of dialysis, alkalinization, and folate substitution) which could affect the outcomes of methanol poisoning and the character of long-term visual sequelae. Further, the time range between discharge from hospitals and the follow-up VEP examinations was from 3 to 8 months, making possible additional inter-individual variability in the results of measurements (due to possible regression or progression of visual damage during this period). Nevertheless, the aim of the study was to examine the prevalence of long-term visual sequelae of methanol poisoning in the population of survivors which persist for more than two months after discharge and can be detected by objective measurements. We did not study possible dynamics of these changes and possible effects of different treatment modalities on the outcome.

Finally, substantial part of the survivors of methanol poisoning did not admit participation on the follow-up examination; therefore, the selection bias is possible with less severely affected patients participating in the study and the real prevalence of long-term visual sequelae of acute methanol poisoning may be even higher than that found in our study.

Despite the limitations, this is the first-ever longitudinal cross-sectional study of long-term visual sequelae after methanol poisoning using a defined protocol and advanced technology to identify and objectively characterize not only the clinical, but also the subclinical lesions of retina, the optic nerve, and the basal ganglia. All patients were examined according to the same standardized protocol including complete ophthalmologic and neurological examinations, as well as biochemical and toxicological tests to limit influence of other confounders.

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Declaration of interests

The authors report no declarations of interests.

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Progressive Chronic Retinal Axonal Loss Following Acute Methanol-induced Optic Neuropathy: Four-Year Prospective Cohort Study

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• PURPOSE: To study the dynamics and clinical determinants of chronic retinal nerve fiber layer thickness (RNFL) loss after methanol-induced optic neuropathy.
• DESIGN: Prospective cohort study.
• METHODS: All patients underwent complete ophthalmic evaluation including spectral-domain optical coherence tomography 3 times during 4 years of observation: 4.9 (±0.6), 25.0 (±0.6), and 49.9 (±0.5) months after discharge. PARTICIPANTS: Eighty-four eyes of 42 survivors of methanol poisoning, mean age (standard deviation) of 45.7 (±4.4) years; and 82 eyes of 41 controls, mean age 44.0 (±4.2) years. MAIN OUTCOME MEASURES: Global and temporal RNFL loss.
• RESULTS: Abnormal RNFL thickness was registered in 13 of 42 (31%) survivors of methanol poisoning and chronic axonal loss in 10 of 42 (24%) patients. Significant decrease of global/temporal RNFL thickness during the observation period was found in the study population compared to the controls ($P < .001$). The risk estimate of chronic global RNFL loss for arterial blood pH < 7.3 at admission was 11.65 (95% confidence interval 1.91–71.12) after adjusting for age and sex. The patients with chronic axonal degeneration demonstrated progressive visual loss in 7 of 10 cases. The patients with abnormal RNFL thickness had magnetic resonance signs of brain damage in 10 of 13 vs 8 of 29 cases with normal RNFL thickness ($P = .003$). Signs of brain hemorrhages were present in 7 of 13 patients with abnormal RNFL thickness vs 5 of 29 cases with normal RNFL thickness ($P = .015$).
• CONCLUSIONS: Methanol-induced optic neuropathy may lead to chronic retinal axonal loss during the following years. Arterial blood pH on admission is the strongest predictor of chronic RNFL thickness decrease. Chronic retinal neurodegeneration is associated with the progressive loss of visual functions and necrotic brain lesions.

METHANOL IS A WIDELY USED INDUSTRIAL SOLvent and is one of the main causes of toxic optic neuropathy. Methanol poisonings present a serious problem owing to the high mortality and prevalence of health sequelae in survivors.[1–3] In the treatment of poisoning, the timely blocking of methanol oxidation by the alcohol dehydrogenase is crucial.[4,5] Accumulation of the toxic metabolite formic acid leads to the inhibition of mitochondrial cytochrome c oxidase, an impairment of oxygen utilization, a depletion of ATP in affected cells, and metabolic acidosis.[6,7]

The retinal ganglion cells and their axons are selectively vulnerable to histotoxic hypoxia caused by formic acid.[8,9] Clinical symptoms of methanol-induced optic neuropathy range from blurred vision, decreased visual acuity, photophobia, and altered visual fields to complete blindness. The recovery of visual disturbances occurs typically within 2–3 weeks. Nevertheless, visual field deficits, central and centrocecal scotomata, color vision abnormalities, and visual acuity loss may persist in up to 40% of survivors.[10,11] New or unrecognized visual disturbances may be revealed during the following years.[12] The visual outcome in survivors of acute methanol poisoning is hardly predictable.[13]

Knowledge of the character of damage to ocular retina, the timely detection of visual sequelae, and understanding the determinants of chronic changes of the visual system are crucial both for assessment of the treatment effectiveness and the prediction of visual loss and the need for

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special equipment to improve the quality of life of the patients. However, prospective cohort studies of long-term visual sequelae of methanol-induced optic neuropathy, with the series of ophthalmologic examinations, optical coherence tomography (OCT) of retinal nerve fiber layer (RNFL) thickness, visual evoked potentials (VEP), and magnetic resonance imaging (MRI) of the brain, are absent.

In this study, we report the data based on the mass methanol poisoning outbreak in the Czech Republic in 2012, with a total of 139 cases of poisoning and more than 50 deaths.[14] In 2013, we performed the cross-sectional study of the prevalence and character of visual damage in the survivors of poisoning after discharge from hospital.[10] During the following 4 years, we studied the dynamics of structural changes of ocular retina and visual functions in this cohort.

METHODS

• **STUDY DESIGN AND SETTING:** This prospective cohort study adhered to the tenets of the Declaration of Helsinki. The study and the informed consent were approved prospectively by the General University Hospital Ethics Committee in Prague, Czech Republic. The study included all patients with confirmed acute methanol poisoning treated in 2012.[14] The clinical and laboratory data at admission were collected prospectively. The data on hospital treatment and outcomes were collected from hospital discharge reports.

The follow-up clinical examinations were performed in the General University Hospital in Prague 3 times during the study period: 4.9 (±0.6) months, 25.0 (±0.6) months, and 49.9 (±0.5) months after discharge from hospital (mean with standard deviation).

• **SELECTION OF PARTICIPANTS AND TREATMENT:** To identify the cases to include in the study, mandatory reporting on all cases of hospital admission with acute methanol poisoning had been established. All patients hospitalized with confirmed diagnosis were eligible for this study. Patients who died out of hospital or during hospitalization were excluded.

For the control group, healthy subjects of the same age and ethnicity and with a history of chronic alcohol abuse were recruited. Exclusion criteria for the controls were intraocular pressure ≥ 22 mm Hg or glaucoma in either eye; evidence of a reproducible visual field (VF) defect (pattern standard deviation significant at the <5% level or abnormal glaucoma hemifield test result) in either eye, as measured with a Medmont automated perimeter M700 (Medmont International Pty Ltd, Vermont, Australia); unreliable VFs (false-positive or false-negative rates > 15% or fixation losses > 20%); any pattern of loss consistent with ocular disease; intraocular surgery in the eye (except cataract or refractive surgery, if performed more than 1 year before testing); best-corrected visual acuity (VA) worse than 20/32 on the Early Treatment Diabetic Retinopathy Study scale; evidence of diabetic retinopathy, diabetic macular edema, or other vitreoretinal disease in either eye; evidence of optic nerve or RNFL abnormality in either eye; and use of a photosensitizing agent within 14 days.

The patients with methanol poisoning were treated in accordance with the practice guidelines for the treatment of methanol poisoning.[15] Ethanol or fomepizole were used as antidotes.[16,17] Hemodialysis was applied to eliminate formic acid and methanol and to correct acidemia.[18,19] Folates were administered to substitute the internal pool.[20]

• **CLINICAL EXAMINATIONS AND LABORATORY ANALYSES DURING HOSPITAL TREATMENT:** The clinical examination protocol included ocular examination with standard ophthalmologic tests, computed tomography (CT) or MRI of the brain, and standard neurologic examination. Patients were considered to have visual sequelae of acute methanol poisoning if the symptoms of toxic optic neuropathy were documented on admission and/or during hospitalization, with pathologic findings of visual acuity, perimeter, color vision, contrast sensitivity, and persisting lesions on funduscopy upon discharge from hospital. Similarly, patients were considered as having central nervous system sequelae of poisoning if symmetrical necrosis and hemorrhages of the basal ganglia and subcortical white matter compatible with the diagnosis of acute methanol poisoning were present on CT or MRI of the brain.

• **FOLLOW-UP CLINICAL EXAMINATION PROTOCOL:** The follow-up clinical examination protocol has been described in detail in our previously published studies.[10,21,22] The study protocol included complete ophthalmologic evaluation and standard ophthalmic tests, including VA measurement, slit-lamp examination, intraocular pressure measurement, fundus examination, color vision, visual fields, spectral-domain OCT (SD-OCT) with RNFL, VEP, MR of the brain, neurologic and neuropsychological examinations, biochemical tests (electrolytes, glucose, glycohemoglobin, albumin, pre-albumin, renal and hepatic tests, cholesterol, lipids, thyroid-stimulating hormone [TSH], vitamins B_{12} and B_1, carbohydrate-deficient transferrin, ethyl glucuronide in urine), and standardized questionnaire forms. The examiners were masked to the results of measurements, severity of poisoning, clinical course, treatment measures, and clinical outcomes.

RNFL thickness was measured on the SD-OCT Spectralis Tracking Laser Tomography (Heidelberg Engineering GmbH, Heidelberg, Germany; software version 5.8.3) and compared to the normative database and to values in the control group. The results of global, nasal, and temporal RNFL measurements performed 3 times during the 4 years

TABLE 1. Demographic and Laboratory Data of the Study Population and the Control Group

	Age, Years	Chronic Alcohol Abuse	Glucose, mmol/L	GlycHbA1, mmol/mol	Creatinine, μmol/L	Cholesterol, mmol/L	TSH, mIU/L	Lipids, mmol/L	Vitamin B_1, μg/L	Vitamin B_{12}, μg/L	GGT, μkat/L
Study population (n = 42)	45.7 ± 4.4	23 (55%)	5.2 ± 0.3	33.9 ± 2.1	79.5 ± 4.5	5.4 ± 0.4	2.3 ± 0.3	1.5 ± 0.3	60.8 ± 5.5	394 ± 51	1.9 ± 0.8
Control group (n = 41)	44.0 ± 4.2	24 (59%)	4.6 ± 0.3	36.0 ± 1.5	76.0 ± 9.4	4.6 ± 0.4	2.1 ± 0.6	1.6 ± 1.0	45.7 ± 4.2	420 ± 110	0.6 ± 0.3
P	.729	.426	.004*	.102	.507	.008*	.554	.859	.001*	.687	.006*

GGT = gamma-glutamyltransferase; TSH = thyroid-stimulating hormone; GlycHbA1 = glycated hemoglobin A1.
P < .05 was considered significant (asterisks).
Data are presented as mean ± standard deviation or n (%).

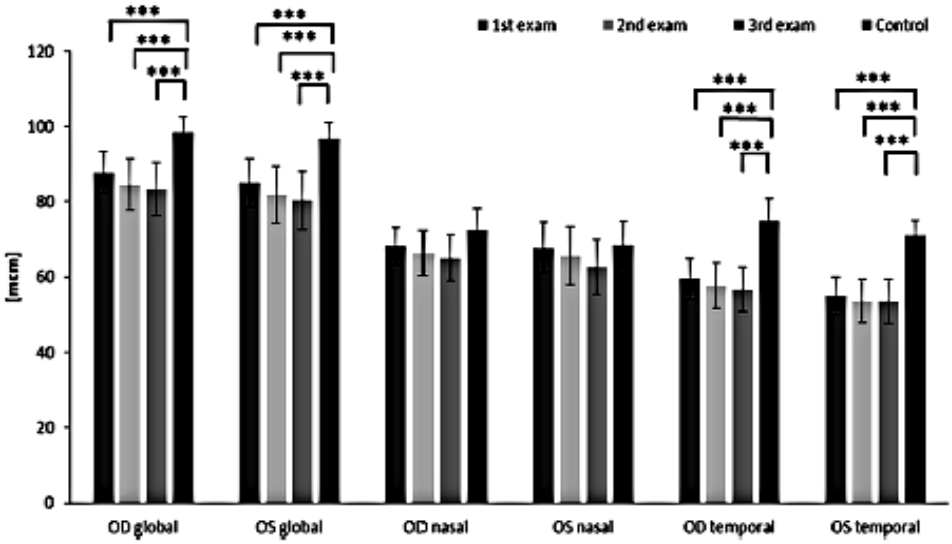

FIGURE 1. Retinal nerve fiber layer (RNFL) thickness measurements in the study population (n = 42) during the observation period vs in the control group (n = 41). ***P < .001. Clinical examinations 4.9 ± 0.6 months (1st exam), 25.0 ± 0.6 months (2nd exam), and 49.9 ± 0.5 months (3rd exam) after discharge from hospital (mean ± standard deviation).

of study were compared to detect significant loss of RNFL thickness during the follow-up period. The criterion for the significant abnormal loss of RNFL was the loss of at least 2 μm in all segments and/or loss of at least 4 μm in 1 (mostly temporal) segment of the retina detected on the second and third examination.

The VEP examination was performed on a 2-channel TruTrace 4 (Alien technik s.r.o., Hronov, Czech Republic) device. Monocular full-field checkerboard pattern-reversal stimulation was used, with the reversal frequency of 1.5 c/s, angular size of the monitor 6 × 5 degrees from the fixation point, angular size of checkerboard squares 40′ (arcmin). Luminance of the white and black squares was 84 cd/m^2 and 57 cd/m^2, respectively. Bandwidth of the amplifier was 1 Hz to 1 kHz; the evoked response was registered from the Oz-Fz derivation. At each eye, the examination was performed twice in order to check reproducibility of the evoked complex. We evaluated the latency of wave P1 and the amplitude of N1P1. Four criteria of abnormality were chosen: (1) missing evoked response, (2) wave P1 latency > 117 ms, (3) interocular difference of wave P1 latencies > 6 ms, and (4) amplitude of evoked complex < 3 μV. The result was categorized as abnormal if at least 1 of the above-mentioned 4 criteria was fulfilled.

The best-corrected visual acuity (BCVA) was established on standard Snellen charts at a distance of 6 m (20 ft). The visual acuity was considered pathologic if worse than 6/6. The visual field was examined by means of static

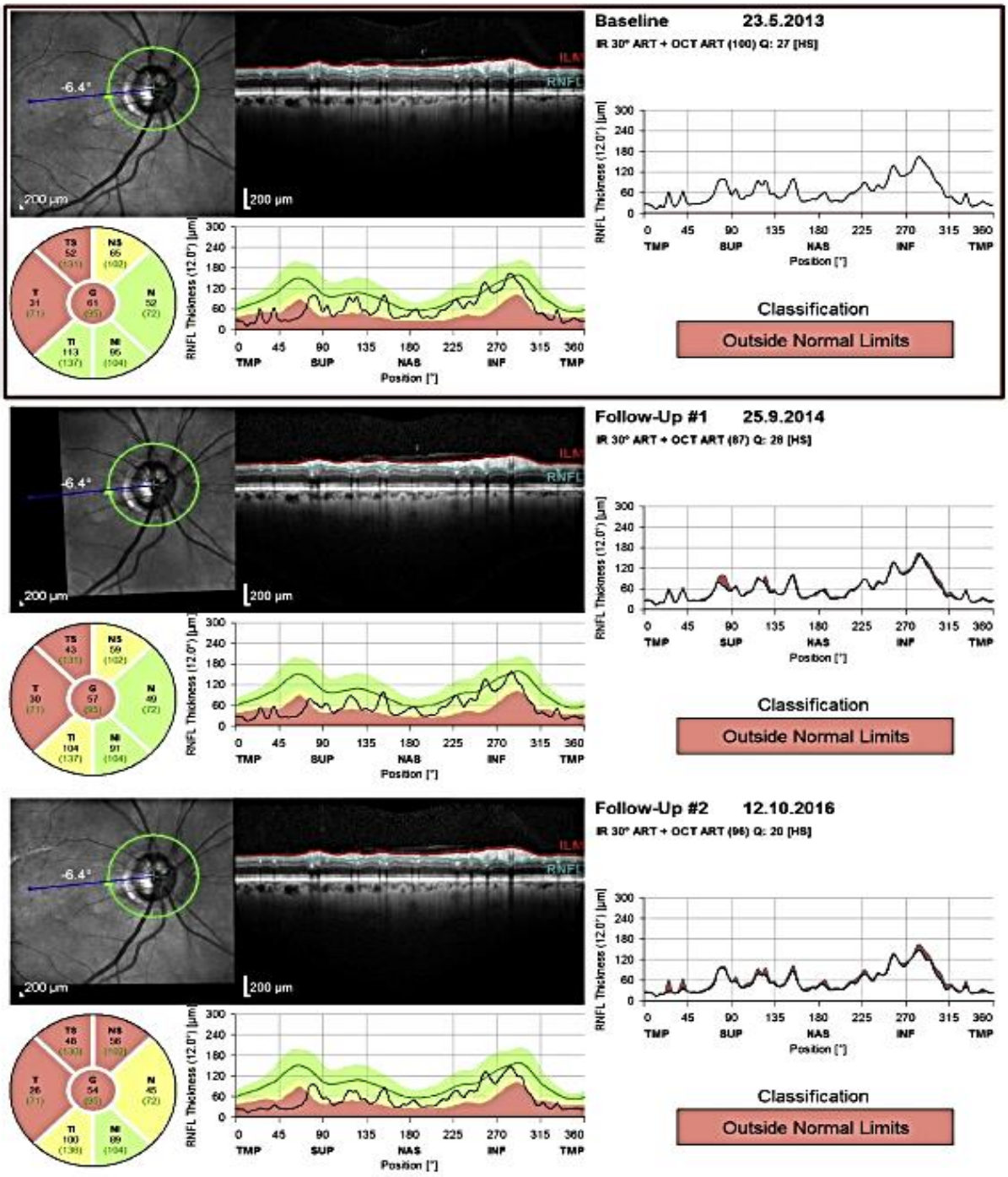

FIGURE 2. Dynamics of individual retinal nerve fiber layer decrease during the observation period.

perimetry (Medmont perimeter M700 automated perimeter; Medmont International Pty Ltd, Vermont, Australia; neurological test, threshold strategy). The findings were considered pathologic if there were any defects in the visual field. The color vision was examined by means of Lanthony's 15-D test (Richmond Products, Albuquerque,

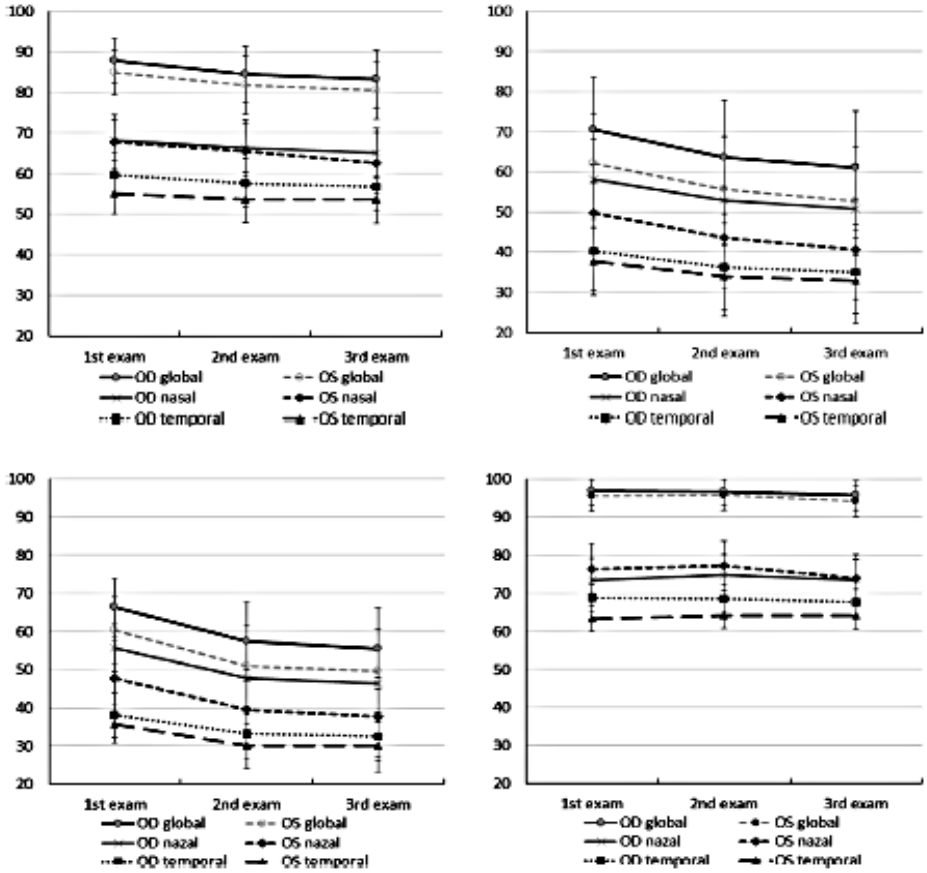

FIGURE 3. Dynamics of retinal nerve fiber layer (RNFL) decrease during four years after acute methanol poisoning in the total study population (Top left), and in patients with severe methanol poisoning (Top right), with abnormal (Bottom left) and normal/borderline (Bottom right) RNFL measurements on the first examination. Clinical examinations 4.9 ± 0.6 months (1st exam), 25.0 ± 0.6 months (2nd exam), and 49.9 ± 0.5 months (3rd exam) after discharge from hospital (mean ± standard deviation).

TABLE 2. Demographic, Toxicologic, and Biochemical Parameters in Patients With Versus Without Significant Chronic Retinal Nerve Fiber Layer Decrease During the Study Period

	Age, Years	MetOH, mg/L	EtOH, mg/L	pH	Creatinine, μmol/L	Glucose, mmol/L	Lactate, mmol/L	Formate, mg/L	Vitamin B_1, μg/L	Vitamin B_{12}, μg/L	GGT, μkat/L
RNFL decrease (n = 10)	50.0 ± 9.2	1950 ± 980	73 ± 95	7.00 ± 0.19	131 ± 32	11.4 ± 4.5	6.0 ± 3.4	620 ± 460	68 ± 18	480 ± 170	0.9 ± 0.6
No RNFL decrease (n = 32)	44.4 ± 5.3	1250 ± 550	280 ± 180	7.22 ± 0.07	83.3 ± 9.1	7.2 ± 1.0	2.4 ± 1.0	600 ± 170	57.8 ± 5.4	413 ± 65	1.1 ± 0.4
P	.290	.201	.042*	.005*	.010*	.078	.053	.859	.272	.384	.444

EtOH = ethanol; GGT = gamma-glutamyltransferase; MetOH = methanol; RNFL = retinal nerve fiber layer.
P < .05 was considered significant (asterisk).
Data are presented as mean ± standard deviation.

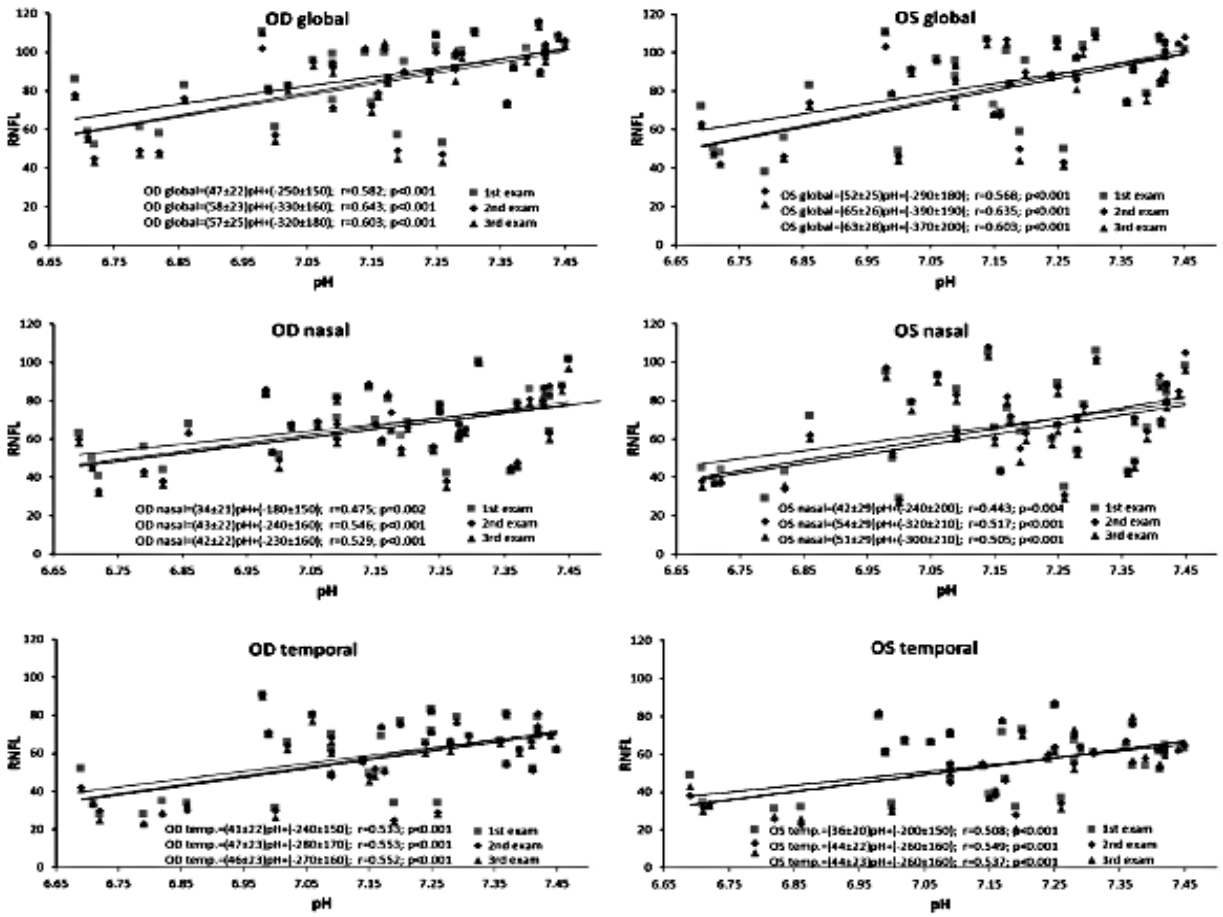

FIGURE 4. Association of global retinal nerve fiber layer (RNFL) thickness (Top, left and right) and RNFL thickness in nasal (Middle, left and right) and temporal segments (Bottom, left and right) measured on 3 consecutive examinations during the study period with arterial blood pH on admission to hospital with acute methanol poisoning. Clinical examinations 4.9 ± 0.6 months (1st exam), 25.0 ± 0.6 months (2nd exam), and 49.9 ± 0.5 months (3rd exam) after discharge from hospital (mean ± standard deviation).

New Mexico, USA). The finding was considered normal up to 3 crossings, borderline with 3–7 crossings, and pathologic with more than 7 crossings. The contrast sensitivity was examined by means of Pelli-Robson contrast sensitivity test (Clement Clarke International Ltd, Essex, UK). The finding was considered normal (1.35 and better), borderline (1.20–1.05), or pathologic (worse than 1.05). The fundus (posterior pole of the eye) was examined by means of biomicroscopy on the slit lamp with the +78 diopter lens (Volk-lens type). The finding was considered pathologic if any related pathology of the optic nerve head and/or the adjacent retina was present.

All patients underwent brain MRI on a Gyroscan Phillips 1.5 T system (Philips, Amsterdam, Netherlands) with the following protocol: axial T2-weighted image (T2WI) with slice thickness (THK) 6.0/0.6 mm through the whole brain, with parameters: repetition time (TR) 4241 ms, time to echo (TE) 100 ms, flip angle (FA) 90 degrees, FLAIR (fluid-attenuated inversion recovery): TR 11 000 ms, TE 140 ms, inversion time (TI) 2800 ms, FA 90 degrees, T1-weighted image (T1WI): TR 569 ms, TE 15 ms, FA 69 degrees, T2WI-fast field echo: TR 665 ms, TE 23 ms, FA 18 degrees, single-shot diffusion-weighted image: TR 2901 ms, TE 75 ms, FA 90 degrees, T1WI after administration of gadolinium and in coronal images centered to the orbital region T2WI with suppression of fat (SPIR): TR 5506 ms, TE 100 ms, FA 90 degrees.

• **CALCULATIONS AND DATA ANALYSIS:** Basic descriptive statistics (mean, median, confidence interval [CI], standard deviation [SD], skewedness, and kurtosis) were computed for all variables, which were subsequently tested for normality using the Kolmogorov-Smirnov test. A χ^2 test was used to compare frequency counts of demographic and

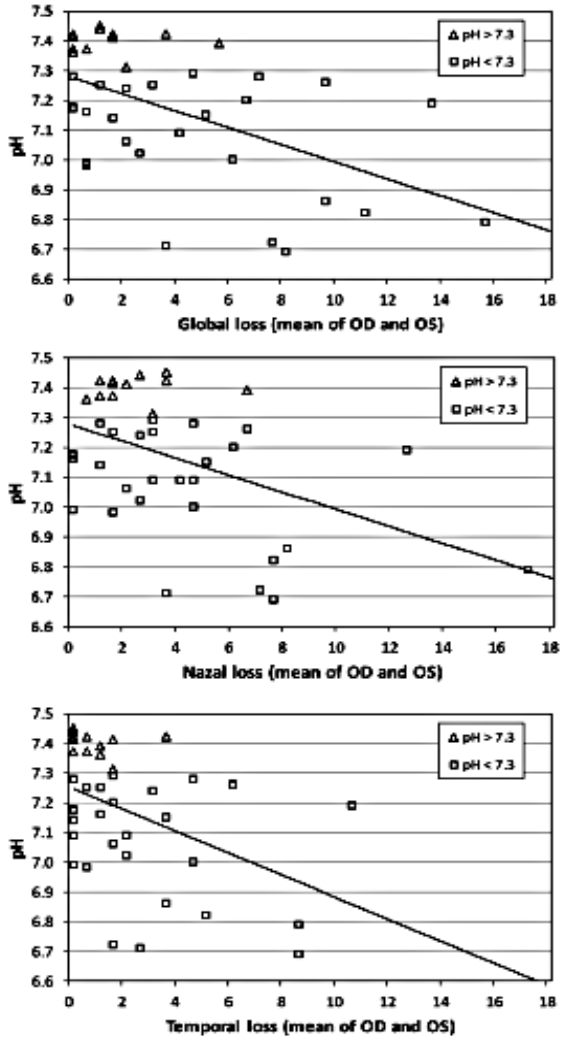

FIGURE 5. Association of the dynamics of global (Top), nasal (Middle), and temporal (Bottom) chronic retinal nerve fiber layer loss during 4 years of observation and arterial blood pH on admission to hospital with acute methanol poisoning. Clinical examinations 4.9 ± 0.6 months (1st exam), 25.0 ± 0.6 months (2nd exam), and 49.9 ± 0.5 months (3rd exam) after discharge from hospital (mean ± standard deviation).

clinical categorical variables. The bivariate relationship was assessed using a Pearson correlation coefficient. A linear mixed-effects model was used to examine the relationship of demographic, clinical, and laboratory parameters with global RNFL thickness and loss during the study period as the dependent variables. The independent variables included in the model were age, sex, severity of metabolic acidosis (arterial blood pH), acute serum concentrations of methanol and ethanol on admission, the follow-up serum glucose, vitamins B_1 and B_{12}, and TSH measured during the study period. The receiver operating characteristic (ROC) analysis was performed in order to identify potentially useful cut-off score of arterial blood pH for prediction of significant RNFL loss. Statistical significance was set at $P < .05$. Statistical documentation was performed in Excel (Microsoft, Redmond, Washington, USA), and the formal calculations were produced in QC Expert software 3.1 (Trilobyte, Pardubice, Czech Republic) and in IBM SPSS version 23.0 (IBM Corp, Armonk, New York, USA).

RESULTS

• **DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE PATIENTS:** A total of 108 patients had been treated in hospital during the methanol poisoning outbreak, of which 84 survived and were invited to participate in a study. Fifty-five patients (65%) with an age of 46.7 ± 3.6 years (mean ± standard deviation), including 46 male and 9 female subjects, agreed to and participated in at least 1 clinical examination.

During the study period, 8 patients died before the third clinical examination, and 5 patients had fewer than 3 examinations, as they joined the study late. These 13 patients were excluded from analysis of the dynamics of chronic retinal axonal loss. Therefore, 42 patients, 34 male and 8 female, participated in 3 consecutive clinical examinations during the 4-year period. The control group of 41 patients underwent clinical examination according to the same investigation protocol. The study analyzed 84 eyes from survivors of acute methanol poisoning and 82 eyes from controls.

The demographic and laboratory data of both groups are presented in Table 1. The age and number of subjects with a confirmed diagnosis of chronic alcoholism were comparable between the 2 groups. The study population had higher mean serum glucose owing to 5 patients with type 2 diabetes mellitus being treated with oral antidiabetics. The mean concentration of serum cholesterol, but not lipids, was higher in the study population compared to the control group. The mean concentration of vitamin B_1 was higher in the study population compared to the controls; no cases of hypovitaminosis B_1 and B_{12} were registered in either group.

On admission to hospital, acute serum methanol concentration in the study population was 1.43 ± 0.47 g/L, arterial blood pH 7.17 ± 0.07, base deficit 16.50 ± 3.50 mmol/L, and formic acid 0.60 ± 0.15 g/L (mean ± standard deviation). Half of the patients had severe metabolic acidosis on admission, 10 patients were admitted in a coma, and 20 patients presented subjective signs of visual damage ranging from blurred vision to complete blindness (3 cases).

• **DYNAMICS OF CHRONIC RETINAL NERVE FIBER LAYER THICKNESS DECREASE:** The results of 3 consecutive RNFL measurements during 4 years of observation are presented in Figure 1. A significant decrease of global RNFL thickness with the most remarkable loss in the

TABLE 3. Final Mixed-Effect Model Predicting Changes of Global Retinal Nerve Fiber Layer Thickness During 4 Years of Observation

Parameter*	Estimate	Standard Error	df	t	Significance	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	87.47	3.52	24.94	24.83	.000*	80.22	94.73
Time	−0.75	0.12	32.04	−6.12	.000*	−0.99	−0.50
OD/OS difference	1.35	0.37	40.64	3.63	.001*	0.60	2.11
Sex	−0.74	3.80	24.90	−0.19	.848	−8.57	7.09
Age	−3.38	3.10	24.99	−1.09	.286	−9.76	3.00
pH	9.66	3.36	25.03	2.88	.008*	2.74	16.57
Time*pH	0.48	0.12	33.34	3.99	.000*	0.24	0.73
MetOH	−3.94	4.24	24.95	−0.93	.361	−12.67	4.79
EtOH	1.87	2.98	25.06	0.63	.536	−4.26	8.00
Glucose	−0.33	0.48	99.62	−0.70	.487	−1.28	0.61
B_1_1st	−0.29	3.30	25.02	−0.09	.931	−7.08	6.51
B_1_2nd	−5.81	2.91	25.00	−2.00	.057	−11.81	0.18
TSH	0.02	0.16	80.13	0.10	.917	−0.30	0.33
B_{12}_1st	−2.63	2.77	24.90	−0.95	.351	−8.33	3.07
B_{12}_2nd	−0.31	5.35	24.95	−0.06	.954	−11.34	10.71
B_{12}_3rd	0.08	5.38	24.95	0.01	.988	−11.01	11.17

$P < .05$ (asterisk) was considered as significant.

*Parameters are defined as follows: df = degrees of freedom; t = hypothesis test statistic value; Time = time from methanol exposure to clinical examination; OD/OS difference = interocular difference in the mean global retinal nerve fiber layer thickness; pH = arterial blood pH on admission; Time*pH = interaction effect of arterial blood pH and time from methanol exposure to clinical examination; MetOH = serum methanol concentration; EtOH = serum ethanol concentration; Glucose = serum glucose concentration; B1 = serum vitamin B_1 concentration; B_{12} = serum vitamin B_{12} concentration; TSH = serum thyroid-stimulating hormone concentration; 1st, 2nd, 3rd = clinical examinations 4.9 ± 0.6 months, 25.0 ± 0.6 months, and 49.9 ± 0.5 months after discharge from hospital (mean ± standard deviation).

temporal segments was observed in the study population compared to the controls. An example of individual RNFL dynamics during the observation period is demonstrated in Figure 2. The rate of RNFL decrease was significantly higher than the rate of age-related physiological decrease (Figure 3, Top left). The highest rate of axonal loss was seen in the most severely poisoned patients (Figure 3, Top right) with metabolic acidosis upon admission. These patients had very low or negative serum ethanol concentrations on admission, along with lower arterial blood pH and higher serum creatinine, compared to the group without a significant decrease of RNFL during the study period (Table 2). The patients with abnormal RNFL thickness found on the first examination had the most significant rate of axonal loss during the period between the first and second rounds of examination (Figure 3, Bottom left). Finally, patients with normal and borderline RNFL thickness measured at the first examination demonstrated no negative dynamics of RNFL thickness during the 4 years of observation (Figure 3, Bottom right).

The interocular difference in mean RNFL thickness in the study population was significant for global and temporal (but not nasal) segments for all 3 rounds of measurements and it was taken into account in the multivariate model as the parameter "OD/OS RNFL thickness difference." No interocular difference in the mean rate of global and temporal axonal loss was seen in the study population.

Arterial blood pH on admission, the indicator of severity of metabolic acidosis, was the laboratory parameter demonstrating the most significant association with RNFL thickness (Figure 4) and the dynamics of chronic axonal loss (Figure 5). In bivariate models, a significant association of RNFL thickness was found with age, sex, serum methanol, ethanol, formate, lactate, and glucose on admission, and with serum vitamin B_1 and B_{12} concentrations measured during the observation period (all $P < .05$). No associations of RNFL thickness were present with serum glucose, glycated hemoglobin, cholesterol, lipids, TSH, and other biochemical laboratory parameters measured during the observation period.

In therapeutic interventions, the association was present between pre–hospital administration of ethanol as a "first aid" antidote and higher global RNFL thickness ($r = 0.388$ and $r = 0.355$; $P = .010$ and $P = .030$ for OD and OS, correspondingly), as well as lower dynamics of chronic axonal loss ($r = -0.396$ and $r = -0.406$; $P = .013$ and $P = .010$ for OD and OS, correspondingly). The patients treated in hospital with intermittent hemodialysis had a lower rate of chronic axonal loss than those treated with continuous modalities of renal replacement therapy ($r = 0.473$ and $r = 0.367$; $P = .002$ and $P = .017$ for OD and OS, correspondingly). No associations between RNFL thickness, dynamics of axonal loss, and other treatment modalities (hospital administration of ethanol

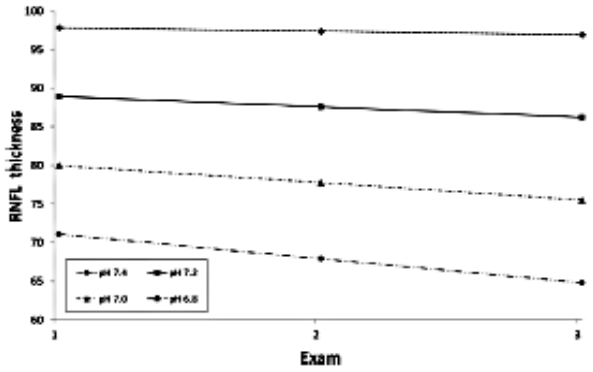

FIGURE 6. Model prediction of global retinal nerve fiber layer (RNFL) thickness decrease during the observation period depending on arterial blood pH on admission adjusted for other independent parameters. Mean Fixed Predicted Values = mean global RNFL thickness, μm; pH = arterial blood pH measured on admission to hospital; Time = clinical examinations 4.9 ± 0.6 months (1st exam), 25.0 ± 0.6 months (2nd exam), and 49.9 ± 0.5 months (3rd exam) after discharge from hospital (mean ± standard deviation).

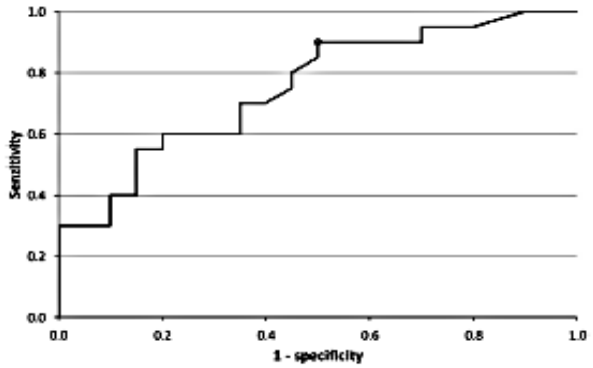

FIGURE 7. Receiver operating characteristic curve predicting global retinal nerve fiber layer thickness loss during the years following methanol-induced toxic optic neuropathy depending on arterial blood pH on admission.

vs fomepizole as an antidote, folate substitution, and others) were found.

For the mixed model regression analysis of the dynamics of global RNFL thickness loss during the study period, the following parameters were included in the model as predictors: age, sex, time after methanol exposure, OD/OS RNFL thickness difference, acute laboratory parameters on admission (arterial blood pH, serum methanol, ethanol, and glucose), vitamins B_1 and B_{12}, and TSH measured during the observation period. The model predicting mean global RNFL thickness during 4 years after acute methanol-induced optic neuropathy for different values of arterial blood pH on admission after adjusting for other independent variables used for multivariate mixed regression analysis (Table 3) is presented in Figure 6.

The ROC curve predicting global RNFL thickness loss during the observation period depending on arterial blood pH on admission after adjusting for age and sex with cut-point of pH 7.3 is presented in Figure 7. The area under the curve for this model is 0.753 ± 0.076 ($P = .006$), the sensitivity is 90%, and the specificity is 50%. For patients with severe acidemia, the lower cut-point of pH 7.16 provides a sensitivity of 60% and specificity of 80%. The risk estimate of significant chronic global RNFL loss (more than 2 μm) during the years after acute methanol-induced optic neuropathy for the patients with arterial blood pH under 7.3 was odds ratio (OR) 9.0 (95% CI 1.64–49.47, $P < .05$) and OR 11.65 (95% CI 1.91–71.12) after adjusting for age and sex.

• **DYNAMICS OF RETINAL NERVE FIBER LAYER THICKNESS DECREASE, P1 LATENCY PROLONGATION, AND N1P1 AMPLITUDE OF VISUAL EVOKED POTENTIALS:** On the first examination, the abnormally prolonged latency of wave P1 of VEP was observed in 18 right eyes (43%) and 21 left eyes (50%), including 5 right eyes and 4 left eyes with nonmeasurable potential. Significant tendency to latency P1 shortening with the most remarkable changes between the first and second examinations was observed with return of the mean latency to the values comparable with those measured in the control group. No significant changes in the N1P1 amplitude of VEP have been registered during the observation period.

The correlations between global and temporal RNFL thickness, the latency P1, and the N1P1 amplitude of VEP measured during the observation period are presented in Table 4. The negative correlation between the P1 latency and RNFL thickness (ie, the longer P1 latency, the thinner RNFL) was the strongest at the first examination; was weaker, but still significant, at the second examination; and finally became insignificant at the third examination. The gradual loss of significance of correlation reflected the process of the advancing remyelination of the optic nerve fibers.

The positive correlation between RNFL thickness and the N1P1 amplitude of VEP (ie, the lower N1P1 amplitude, the thinner RNLF) demonstrated the opposite tendency: it was stronger on the third examination compared to the first one (Table 4). The dynamics of the global RNFL decrease were positively correlated with the P1 latency of VEP measured on the first 2 examinations: the rate of chronic axonal loss was higher in the patients with longer P1 latency owing to more severe acute demyelination of the optic nerve (OD: $r = 0.392$ and $r = 0.396$; OS: $r = 0.322$ and $r = 0.386$ for the first and second examinations, all $P < .05$). Finally, the rate of chronic axonal loss negatively correlated with the N1P1 amplitude of VEP measured on all 3 rounds of clinical examinations. The patients with lower amplitude of VEP had a higher rate of chronic axonal loss.

TABLE 4. Correlations of Retinal Nerve Fiber Layer Thickness, Latency P1, and Amplitude N1P1 of Visual Evoked Potentials

		OD Global RNFL 1st Exam	OD Global RNFL 2nd Exam	OD Global RNFL 3rd Exam	OS Global RNFL 1st Exam	OS Global RNFL 2nd Exam	OS Global RNFL 3rd Exam	OD Temporal RNFL 1st Exam	OD Temporal RNFL 2nd Exam	OD Temporal RNFL 3rd Exam	OS Temporal RNFL 1st Exam	OS Temporal RNFL 2nd Exam	OS Temporal RNFL 3rd Exam
OD P1 1st Exam	Pearson Correlation	−0.424	−0.426	−0.420	−0.468	−0.489	−0.475	−0.540	−0.530	−0.557	−0.508	−0.502	−0.463
	Sig. (2-tailed)	.010*	.010*	.011*	.005*	.002*	.002*	.001*	.001*	.000*	.002*	.002*	.004*
OD P1 2nd Exam	Pearson Correlation	−0.411	−0.438	−0.441	−0.461	−0.506	−0.499	−0.447	−0.469	−0.510	−0.490	−0.492	−0.471
	Sig. (2-tailed)	.014*	.009*	.008*	.005*	.002*	.002*	.007*	.004*	.002*	.003*	.003*	.004*
OD P1 3rd Exam	Pearson Correlation	0.106	0.111	0.105	0.166	0.136	0.133	0.136	0.107	0.127	0.083	0.077	0.083
	Sig. (2-tailed)	.531	.513	.537	.327	.421	.432	.421	.530	.452	.625	.649	.627
OS P1 1st Exam	Pearson Correlation	−0.535	−0.528	−0.548	−0.595	−0.588	−0.604	−0.517	−0.510	−0.554	−0.497	−0.487	−0.503
	Sig. (2-tailed)	.001*	.001*	.000*	.000*	.000*	.000*	.001*	.001*	.000*	.002*	.002*	.001*
OS P1 2nd Exam	Pearson Correlation	−0.521	−0.533	−0.549	−0.457	−0.484	−0.502	−0.463	−0.506	−0.535	−0.408	−0.430	−0.450
	Sig. (2-tailed)	.001*	.001*	.001*	.005*	.003*	.002*	.004*	.002*	.001*	.013*	.009*	.006*
OS P1 3rd Exam	Pearson Correlation	−0.027	−0.034	−0.040	0.029	−0.009	−0.016	0.001	−0.046	−0.026	−0.046	−0.069	−0.065
	Sig. (2-tailed)	.877	.845	.819	.869	.959	.926	.996	.791	.879	.789	.688	.707
OD N1P1 1st Exam	Pearson Correlation	0.273	0.296	0.356	0.393	0.385	0.419	0.461	0.463	0.495	0.505	0.473	0.495
	Sig. (2-tailed)	.107	.080	.033*	.018*	.020*	.011*	.005*	.004*	.002*	.002*	.004*	.002*
OD N1P1 2nd Exam	Pearson Correlation	0.250	0.256	0.321	0.414	0.402	0.434	0.381	0.389	0.438	0.448	0.456	0.468
	Sig. (2-tailed)	.147	.138	.060	.013*	.017*	.009*	.024*	.021*	.008*	.007*	.006*	.005*
OD N1P1 3rd Exam	Pearson Correlation	0.253	0.254	0.312	0.399	0.373	0.400	0.397	0.383	0.432	0.463	0.453	0.457
	Sig. (2-tailed)	.130	.130	.060	.015*	.023*	.014*	.015*	.019*	.008*	.004*	.005*	.004*
OS N1P1 1st Exam	Pearson Correlation	0.410	0.431	0.432	0.428	0.420	0.426	0.291	0.297	0.306	0.320	0.309	0.316
	Sig. (2-tailed)	.011*	.007*	.007*	.007*	.009*	.008*	.076	.070	.062	.050	.059	.053
OS N1P1 2nd Exam	Pearson Correlation	0.309	0.315	0.375	0.428	0.416	0.444	0.412	0.426	0.474	0.435	0.438	0.453
	Sig. (2-tailed)	.066	.061	.024*	.009*	.012*	.007*	.013*	.010*	.003*	.008*	.007*	.006*
OS N1P1 3rd Exam	Pearson Correlation	0.304	0.314	0.370	0.451	0.432	0.458	0.394	0.397	0.450	0.452	0.451	0.470
	Sig. (2-tailed)	.072	.062	.026*	.006*	.009*	.005*	.018*	.017*	.006*	.006*	.006*	.004*

Sig. = significance.

Timing of clinical examinations (Exam) was as follows: 1st, 2nd, and 3rd exam 4.9 ± 0.6 months, 25.0 ± 0.6 months, and 49.9 ± 0.5 months after discharge from hospital (mean ± standard deviation).

$P < .05$ (asterisk) was considered as significant.

TABLE 5. Dynamics of Visual Loss in Patients (N = 10) With Significant Chronic Retinal Nerve Fiber Layer Thickness Decrease

	Patient									
	1	2	3	4[a]	5	6	7	8	9[a]	10
Age/sex	69/M	58/M	61/M	48/M	48/M	34/M	65/M	46/M	33/M	38/M
VA 1st exam	1.0/0.3[b]	0.1/0	0.6/0.6	0.03/0.03	0.6/0.6	1.0/0.8	1.0/0.6	0.06/0.03	0.03/0.01	0.9/0.7
VA 2nd exam	1.0/1.0	0.1/0	06./0.7	0.03/0.03	0.6/0.5	0.9/0.8	1.0/0.8	0.1/0.06	0.08/0.03	0.6/0.6
VA 3rd exam	1.0/1.0	0.08*/0*	0.6/0.6	CF*/0.03*	1.0/0.7	0.9/0.9	0.6*/0.6*	0.15/0.3	0.08/0.03	0.5*/0.4*
Perimeter 1st exam	Nasal inferior defects OU	OD residue paracentral superior/OS impossible	OD constriction/ OS normal	OD/OS constriction in upper and lower fields	OD/OS circular constriction	OD/OS nasal constriction	OD/OS defects nasal and temporal inferior	OD/OS centrocecal scotomata	OD/OS defect in the whole visual fields	Normal
Perimeter 2nd exam	Same	Impossible*	OD constriction/ OS normal	Same	OS progression to the center*	Progression OS to temporal segments*	Same	Same	OD/OS residues nasal and upper fields*	OD/OS low reliability*
Perimeter 3rd exam	Same	Impossible*	OD constriction/ OS normal	OD/OS residues temporal and nasal*	Same	Same	Same	Same	Same	Same
CS 1st exam	N/B[b]	P/ND	N/B	P/P	P/P	B/B	P/P	P/P	ND	B/P
CS 2nd exam	N/N	P/ND	N/B	ND*	B/P	B/B	B/B	B/P	P/ND	B/P
CS 3rd exam	B[b]/N	P/ND	N/B	ND*	P/P	B*/P*	B/B	P/P	P/ND	P*/P*
CV 1st exam	N/N	P/ND	N/N	P/P	N/N	N/B	N/N	B/P	ND	N/N
CV 2nd exam	B/N	P/ND	N/B	ND*	N/N	N/N	N/B	B/B	ND	N/N
CV 3rd exam	B/B	P/ND	N/N	ND*	N*/P*	B*/B*	N*/B*	B/B	P/ND	B*/P*

B = borderline findings; CF = counting fingers; CS = contrast sensitivity; CV = color vision; Exam = examination; N = normal findings; ND = not detectable/measurable; P = pathologic findings; VA = visual acuity (logMAR).

Results are shown as OD/OS.

Asterisk indicates progression.

[a]Because of poor central vision the results of further examinations are not valid.

[b]Cataract.

• **CHRONIC RETINAL NERVE FIBER LAYER THICKNESS DECREASE, DYNAMICS OF VISUAL LOSS, AND BRAIN DAMAGE ON MAGNETIC RESONANCE IMAGING:** The patients with significant chronic retinal axonal loss demonstrated progressing visual loss during the study period in 7 out of 10 cases. The progression of decreased visual acuity was registered in 4 cases, worsening of visual field defects and perimeter constriction in 6 cases, decrease of contrast sensitivity in 3 cases, and worsening of color vision in 5 cases (Table 5). All of the patients with progressing visual loss were male, with ages ranging from 33 to 69 years. Only Patient 7 (Table 5) had type 2 diabetes mellitus treated with metformin. No other relevant somatic or ocular diseases were present in this group of patients.

Magnetic resonance signs of brain damage were present in 18 out of 42 (43%) patients. Bilateral symmetric necrotic lesions of the putamen were found in 16 (38%) patients. In 12 of the 18 patients (67%), hemorrhagic brain lesions were registered, while nonhemorrhagic lesions were found in 6 patients. Pathologic lesions in the optic nerve were found in 3 (7%) patients. The results of the second and third follow-up MRI examinations revealed neither progression nor signs of regression of brain lesions.

Positive association was present between MRI signs of brain damage, brain hemorrhage, and global and temporal chronic retinal axonal loss (all $P < .05$). The prevalence of long-term visual sequelae of poisoning in the group of patients with brain hemorrhagic lesions was higher than in the group without brain hemorrhage and in patients with other nonhemorrhagic brain lesions. The patients with abnormal RNFL thickness had MRI signs of brain damage in 10 (77%) of 13 cases compared to 8 (28%) of 29 cases in the group with normal or borderline RNFL thickness ($P = .003$). Signs of brain hemorrhages were present in 7 (54%) of 13 patients with abnormal RNFL thickness compared to 5 (17%) of 29 cases in the group with normal or borderline RNFL thickness ($P = .015$).

DISCUSSION

THE PREVALENCE OF LONG-TERM VISUAL SEQUELAE OF toxic optic neuropathy caused by exposure to methanol has been shown to be 40%.[10] We found that acute retinal ganglion cell injury was followed by chronic neurodegeneration and progressive axonal loss in up to 25% of the patients. This process was associated with the progressive loss of visual functions. Most of the patients with abnormal RNFL thickness had MRI signs of necrotic brain damage. Arterial blood pH on admission was strongly associated with the degree of acute retinal damage and the rate of chronic axonal loss. Prehospital administration of ethanol and a higher rate of formate elimination and acidemia correction by intermittent hemodialysis were associated with higher RNFL thickness in the study population.

Acute methanol exposure is a life-threatening condition with mortality exceeding 30%–40% and serious central nervous system sequelae; therefore, long-term visual outcomes are often considered "of secondary importance."[23–25] Nevertheless, the visual loss up to complete blindness, central and centrocecal scotomata, and visual field and color vision defects present a serious problem for the survivors.[10–13] In our previous study, we demonstrated that both higher total medical costs and significantly lower quality of life of the patients after discharge from hospital were associated with long-term visual sequelae of poisoning.[26]

Retinal ganglion cells and axons of the optic nerve are the main targets for neurotoxic effects of formic acid; however, chronic changes in the visual system methanol exposure have not been studied. One of the reasons for this is the loss of contact with the survivors after discharge from hospital.[12] In sporadic case reports and small retrospective case series studies, it was demonstrated that both partial recovery and the progression of visual loss could be observed 6–9 months after discharge.[11,13,27]

The Czech Republic methanol mass poisoning outbreak in 2012 provided us the opportunity to perform a cross-sectional study of the prevalence and character of visual damage in the survivors 4.9 ± 0.6 months after discharge from hospital.[10] Peripapillary retinal edema subsides during up to 2 months after acute neuropathy and RNFL measurements immediately after discharge may produce the false result with "normal" RNFL thickness. The collected data were used as the "reference point" for the prospective longitudinal study of the dynamics of RNFL loss and visual function changes in this cohort of patients.

Our results demonstrated that global and temporal RNFL thickness, but not in nasal segments, in the study population was significantly lower compared to the control group of the same age, sex, and alcohol consumption pattern. Taking into account that chronic alcohol abuse is associated with higher rates of RNFL thinning, we recruited sufficient numbers of patients with a confirmed diagnosis of chronic alcoholism to the control group.[28] We registered the process of chronic axonal loss in the patients who survived severe methanol poisoning with high acidemia; the rate of RNFL loss in the study population significantly exceeded the physiological age-related decay.[29]

The fact that the temporal retinal nerve fibers with predominantly small-caliber axons were more vulnerable, while the nasal fibers with more heterogeneous axon population were spared, suggests possible contribution of the mitochondrial dysfunction to the process of chronic neurodegeneration after methanol-induced optic neuropathy.[30,31] In the optic neuropathy with known mitochondrial involvement, Leber hereditary optic neuropathy, the order of RNFL involvement is temporal first, with the nasal segment the last one.[32]

Formate toxicity directly interferes with mitochondrial function: inhibition of cytochrome c oxidase leads to the

high reactive oxygen species (ROS) environment during the acute methanol poisoning.[6,33] Mitochondrial dysfunction leads to excessive production of ROS and toxic aldehydes, with oxidative damage of cellular structures including mitochondrial membranes, release of cytochrome c from the intermembrane space, and apoptosis.[34] The original insult leads to the neuronal degeneration, apoptosis, and death of the smallest nerve fibers, but the other smaller fibers that survived may contain mitochondria with acquired impairment owing to a large number of accumulated mitochondrial DNA (mtDNA) deletions from the high ROS environment during the acute attack. Mitochondrial DNA is particularly susceptible to ROS attack associated with oxidative stress.[35] The persistence of mtDNA damage ultimately leads to mutations in the mitochondrial genome and gives rise to further mitochondrial dysfunction that would produce the delayed reaction of chronic neurodegeneration observed in the study. Other reasons for the continuation of the process long after the metabolic insult resolved may be explained by the "common reservoir" effect described by Pan and associates: the spread of the molecules that signal apoptosis, release of excitotoxic neurotransmitters, and reduction in trophic feedback to the glial cells with the loss of further retinal ganglion cells associated with them are possible elements mediating this phenomenon.[31]

Severe metabolic acidosis is the main prognostic factor of mortality in acute methanol poisoning.[36,37] In our study, the degree of acidemia upon admission was associated with both global and temporal RNFL thickness and with the rate of chronic axonal loss. The dissociation constant of formic acid (pKa) is 3.8; that is, a pH drop by 0.3 would mean a doubling of the undissociated formic acid levels, and hence a significant increase in neurotoxicity, because only undissociated formic acid crosses the neuronal cell membranes.[6,7] A positive serum ethanol concentration prevents formic acid accumulation and acidemia progression and is associated with a better clinical outcome.[5,38] Higher rates of formic acid elimination and acidemia correction during intermittent hemodialysis may be responsible for the association of the mode of dialysis with the rate of chronic axonal loss in our study.[18,39]

Chronic retinal axonal loss might be caused by other pathologic conditions that are unrelated to methanol exposure. Vitamin B_{12} or B_1 deficiency caused by chronic alcoholism or nutritional deficits, thyroid gland hypofunction, or poorly controlled glycemia in diabetes mellitus may cause axonal loss owing to the inadequate restoration of the trophic and protective functions of glia.[40–44] In our study, no association was found between the concentration of serum glucose, TSH, and vitamins B_{12} and B_1 repeatedly measured during the observation period and the dynamics of retinal axonal loss. Only 1 patient with chronic axonal loss had type 2 diabetes mellitus and no patients with B_{12} or B_1 hypovitaminosis and hypothyroidism were present in the group.

The issue of association between structural changes of retina and functional changes of the optic nerve registered by conventional full-field VEP is the subject of debates. There are studies demonstrating a significant association between the results of VEP and RNFL measurements and the studies questioning this association.[45–48] Our results demonstrated that the association depended on the time of measurement. We previously reported that the conductivity of the optic nerve had been restored in more than 80% of patients during the 2 years after acute methanol-induced optic neuropathy.[49] On the other hand, progressing chronic retinal axonal degeneration leads to the strengthening of the association between RNFL thickness and the N1P1 amplitude of VEP.[50] The interesting finding was the association between the amplitude of VEP and the rate of chronic axonal loss: the lower the initial N1P1amplitude (ie, the higher the number of acutely damaged retinal ganglion cells), the higher the rate of chronic axonal degeneration during the following years. The mechanisms of neuroinflammation responsible for chronic neuronal degeneration after traumatic and nontraumatic brain injury may be involved in this process.[51–54]

The progression of visual loss in most patients with chronic retinal neurodegeneration in our study is the issue of concern from the quality-of-life point of view during the years following discharge from hospital.[55] In most of the patients with chronic retinal axonal loss, we registered a further decrease of visual functions limiting them in professional and common life: an impairment of visual acuity, contrast sensitivity, visual field defects, perimeter constriction, and color vision worsening. The optic disc atrophies were diffuse and more pronounced in the temporal segments, without signs of glaucomatous excavation. The visual field defects were not characteristic for the low-tension glaucoma. More often the upper and nasal peripheral fields were involved and the findings were bilateral, mostly asymmetrical. In 1 case, we have found the centrocecal scotoma, but mainly the central vision in the study population was not affected (with the exception of the most severe cases). The paracentral scotomata found in several patients were outside the region characteristic for Bjerrum scotoma. Only 1 of the patients in this group had type 2 diabetes mellitus with normal glucose and glycated hemoglobin measured during the study period. No other somatic or ophthalmologic causes of progressive visual loss were found in these patients. This fact raises the issue of the need for dispensarization and regular ophthalmologic examination of the patients after methanol-induced optic neuropathy.

Finally, the association between chronic retinal neuronal degeneration and MRI findings of the brain damage indicates possible relation between visual and brain sequelae of poisoning. MRI of the brain is not the routine examination in patients with acute methanol intoxication and the number of unrecognized brain lesions is high.[22,24,56] Therefore,

some of these patients with unrecognized necrosis of basal ganglia may develop secondary Parkinsonism later in their life without determination of the direct relationship to methanol exposure. Our study demonstrated that the signs of progressive retinal neuronal degeneration are strongly associated with hemorrhagic necrotic lesions of the brain, which indicates the necessity of MRI examinations and periodic neurologic examinations in this population.

In summary, acute methanol-induced optic neuropathy may lead to chronic retinal axonal loss during the following years. Chronic retinal neurodegeneration leads to the progressive decrease of visual functions. Cases of chronic RNFL loss are associated with hemorrhagic and nonhemorrhagic necrotic brain lesions.

• **STRENGTH AND LIMITATIONS:** There were several limitations to the design of this study. The relatively limited sample size could lead to insufficient power of the study in determining the association of chronic RNFL loss with certain laboratory and clinical parameters measured during hospitalization and the observation period. The study was not controlled with regard to the comorbidity of the patients hospitalized with acute poisoning. Nevertheless, the study population was relatively young; the proportion of patients with comorbidities was low, and the parameters measured during the study allowed us to exclude these comorbidities as possible causes of chronic RNFL loss.

The study was not controlled for the time to hospital presentation and treatment modality (choice of antidote and mode of dialysis, alkalization, and folate substitution), which may have affected the outcome of methanol poisoning and the character of visual outcome.

The aim of the study was to measure the dynamics of RNFL loss, optic nerve function, and visual function in patients who survived methanol poisoning for **4** years after discharge; to compare RNFL thickness with the control group; and to test the associations between the morphologic state of the retina and the key parameters of acute poisoning. Finally, a substantial number of survivors did not participate in the follow-up examinations; therefore, selection bias is possible, with fewer severely affected patients participating in follow-up. Nevertheless, the admission laboratory parameters characterizing the severity of poisoning in the study population demonstrated that half of the patients were severely poisoned and presented subjective signs of visual damage on admission.

Despite these limitations, this is the first prospective cohort study of the dynamics of chronic RNFL thickness decrease in survivors of acute methanol poisoning performed during the **4** years after discharge by applying a standardized clinical examination protocol and advanced technological measurements in the same medical facility.

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11.3. PŘÍLOHA III

Zakharov S, **Nurieva O,** Kotikova K, Urban P, Navratil T, Pelclova D.

Factors predicting optic nerve axonal degeneration after methanol-induced acute optic neuropathy: A two-year prospective study in 54 patients.

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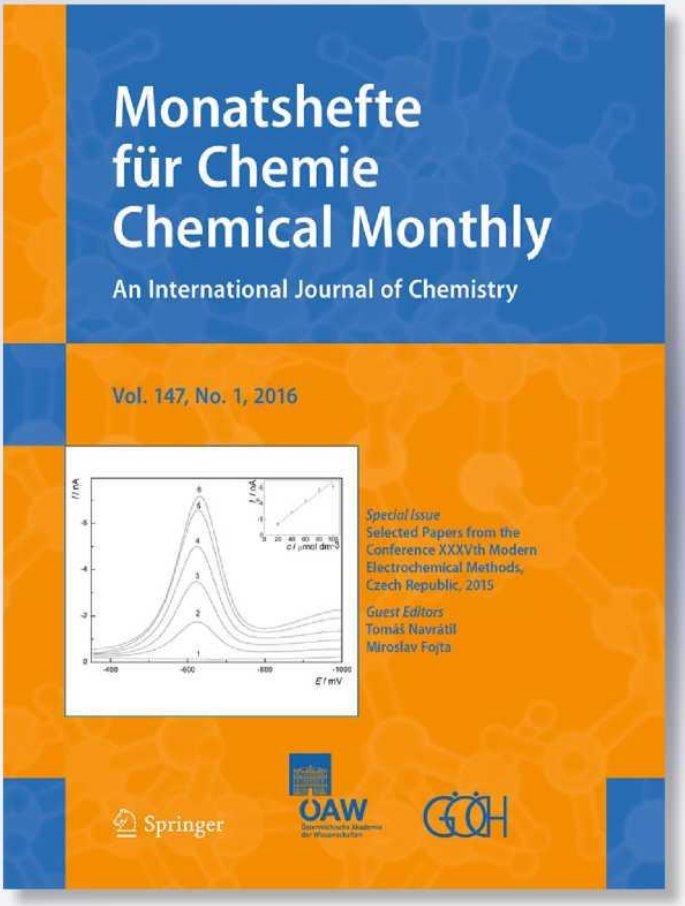

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ORIGINAL PAPER

Factors predicting optic nerve axonal degeneration after methanol-induced acute optic neuropathy: a 2-year prospective study in 54 patients

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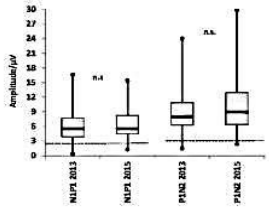
Abstract Prospective cross-sectional study was conducted in 54 patients (mean age 46.7 ± 3.7 years) to determine the character of optic nerve axonal degeneration after acute methanol poisoning. Methanol was measured by a gas chromatographic method with flame ionization detection. Formate was measured enzymatically. Measurement of full-field visual evoked potential with monocular checkerboard pattern-reversal stimulation was performed 3–8 and 24–28 months after discharge. The amplitudes of N1P1 and P1N2 components of evoked response were used for analysis of axonal loss. Altogether, 13 of 50 patients (26 %) had abnormal amplitudes at the first examination (including the patients with nonrecordable amplitudes), and 37 patients had normal amplitudes. Mean N1P1/P1N2 amplitudes for right eyes (REs) were 6.30 ± 1.10/8.70 ± 1.50 μV and for left eyes (LEs) were 6.56 ± 1.00/8.30 ± 1.40 μV. The group with abnormal amplitudes had lower arterial pH ($p = 0.009$), bicarbonate ($p = 0.036$), higher base deficit ($p = 0.005$), glucose ($p = 0.015$), and lactate ($p = 0.018$). At the second examination, insignificant amplitude changes were registered (REs 6.50 ± 1.10/9.80 ± 1.60 μV, LEs 6.40 ± 1.10/9.30 ± 1.60 μV; both $p > 0.05$). In 2 of 44 REs (5 %) and in 4 of 45 LEs (9 %) with 2 consecutive examinations the initially normal amplitudes deteriorated to abnormal values. In 3 of 45 patients (7 %) the abnormal amplitudes deteriorated in both eyes indicating the ongoing process of chronic neuronal degeneration. The dynamics of amplitude deterioration correlated with serum lactate ($r = 0.533$; $p < 0.001$), glucose ($r = 0.462$; $p = 0.005$), and formic acid ($r = 0.380$; $p = 0.046$) on admission to hospital. The correlation was present between the magnetic resonance signs of hemorrhagic brain lesions and the amplitude changes ($r = -0.535$; $p < 0.001$).

Graphical Abstract



Keywords Methanol optic neuropathy · Visual evoked potentials · Axonal degeneration · Visual sequelae of poisoning · Prognosis · Metabolic acidosis

Introduction

Methanol poisonings occur either intentionally through abuse of methanol-containing fluids or attempted suicide or unintentionally through misuse or accident [1–3]. In the

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last decade several mass poisoning outbreaks have been reported as a result of its use as a cheap substitute for ethanol [4–6]. Despite the significant progress made in diagnosis and treatment of acute methanol poisoning, mortality and probability of long-term health sequelae remain high [7, 8]. During the years following discharge from hospital, the possibility of new—or unrecognized—visual and neurological complications in up to 36 % of patients is suggested [9].

Methanol is metabolized by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase, mainly in the liver. Formic acid is the toxic metabolite of methanol responsible for the histotoxic hypoxia by inhibition of cytochrome *c* oxidase in mitochondria [10, 11]. Undissociated formic acid specifically targets optic nerve causing myelin sheath swelling, myelin breakdown, and direct axonal damage [12]. The myelin sheath edema is responsible for the compression-type injury to the nerve fibers (axonal trauma), which in combination with inhibition of mitochondrial function leads to optic nerve conduction deficit, axonal degeneration, and finally neuronal loss with blindness in the most severe cases [13, 14]. With adequate treatment consisting in prevention of toxic metabolite formation by administration of antidotes blocking the ADH enzyme, fomepizole or ethanol, correction of metabolic acidosis with bicarbonate, folate substitution, and rapid elimination of methanol and formic acid by hemodialysis, a complete recovery of visual function is possible within several weeks [15–18]. Nevertheless, serious visual disturbances may persist in 10–30 % of survivors [9, 19].

Several prognostic factors of mortality in methanol poisonings are known including severity of metabolic acidosis, high serum lactate and formate concentrations, hyperglycemia, and negative serum ethanol on admission [20, 21]. However, biochemical and toxicological parameters of poisoning affecting the degree of axonal loss and dynamics of possible chronic degeneration of optic neurons during the years following the acute toxic neuropathy have not been studied. In the retrospective study of Desai et al., the degree of acidosis at presentation appeared to determine final visual acuity [22]. Nevertheless, according to Sanaei-Zadeh et al., long-term visual outcomes in the cases of severe methanol poisoning are hardly predictable [23].

Visual evoked potentials (VEP) are an objective means of measuring the function of visual pathway [24]. The magnitude of evoked response reflects the number of functional afferent fibers reaching the striate cortex which is determined by the severity of axonal degeneration along the visual pathway [25]. Therefore, diminished amplitude of evoked response measured later than 2 months after discharge from hospital in the patients who survived acute methanol poisoning indicates the degree of optic nerve axonal atrophy and neuronal loss. Knowledge of biochemical determinants associated with the degree of axonal degeneration of the optic nerve and objective measures of its dynamics during the years following discharge from hospital are critical in both the prediction of the character of long-term visual sequelae of methanol-induced acute optic neuropathy and in the assessment of the clinical effectiveness of therapeutic interventions.

We examined the prevalence and degree of optic nerve axonal degeneration and its association with laboratory parameters measured on admission to hospitals and within 2-year follow-up clinical examination in a cohort of patients with confirmed acute methanol poisoning. Our hypothesis was that the prevalence and degree of axonal degeneration would be associated with certain biochemical parameters characterizing the severity of acute methanol poisoning.

Results

Demographic characteristics

During the Czech methanol outbreak from September 2012 to August 2014, 137 patients were poisoned and 106 of them were treated in hospital; 83 patients survived methanol poisoning. Of them, 54 patients with mean age 46.7 ± 3.7 years, 45 males and 9 females, were subjected to the follow-up clinical examination 2 times during the study period: 3–8 months and 24–28 months after discharge from hospital (Fig. 1). Five patients only participated in the first clinical examination 3–8 months after poisoning (three of these patients died 1–2 years after acute methanol poisoning, one patient rejected further participation in the study, and contact was lost with the fifth patient). Further, four patients agreed to participate in the study after the first follow-up clinical examination of the population had already been performed and were examined only once, during the second follow-up examination. The results of amplitude measurements in these nine patients were excluded from the analysis of association of the dynamics of amplitude changes during a 2-year period with biochemical and toxicological parameters.

The median ingested volume of toxic spirits was 300 cm^3 (range 100–1500 cm^3) comprising approximately 50 % methanol and 50 % ethanol in different kinds of strong alcoholic beverages with a total alcohol content of around 40 % alcohol by volume (ABV, v/v). In 22 % of the patients the diagnosis was made within 12 h of methanol ingestion, in 66 % within 48 h, and in 6 % later than 48 h (in 6 % it was impossible to determine the time of ingestion reliably). A medical history of chronic alcohol abuse was present in 48 % of the cases. The co-morbidity included 21 cases with hyperlipidemia, 19 cases with

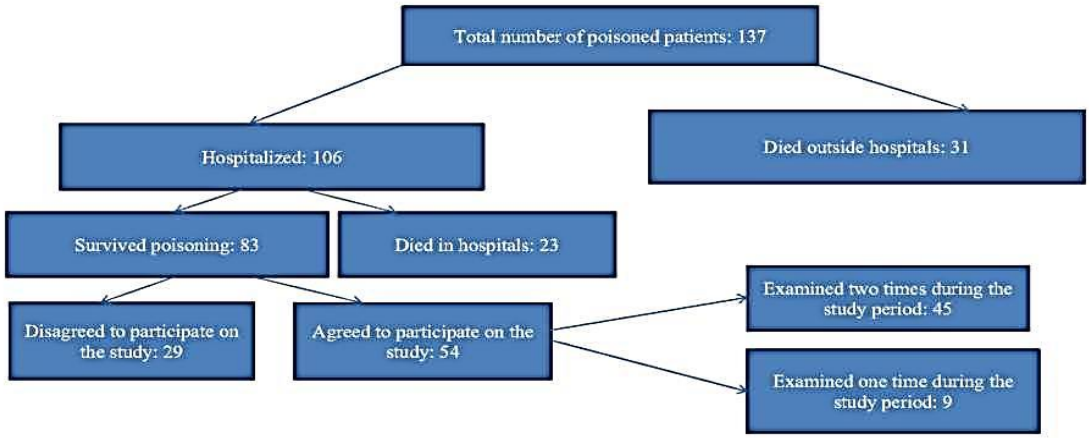

Fig. 1 *Flowchart* of the study design: the outbreak of methanol poisonings in the Czech Republic from September 2012 to August 2014

arterial hypertension, 16 cases with alcoholic hepatopathy, 4 cases with diabetes mellitus (type II in 3 cases, type I in 1 case), 3 cases with myocardial infarction, and 1 case with brain stroke in anamnesis before methanol poisoning; epilepsy, sarcoidosis, psoriasis vulgaris, chronic atrial fibrillation, and ischemic disease of the lower extremities were present each in 1 patient. No patients with multiple sclerosis and other demyelinating and neurodegenerative diseases were present in the study population.

The dynamics of amplitude changes of major components of evoked complex in the 2 years following poisoning

On initial examination at 3–8 months after discharge from hospital, N1P1 and P1N2 amplitudes were measurable for 42 of 50 right eyes (REs) and were abnormally decreased in 6 of 42 (14 %) of them for both measured components of evoked complex (in a further 6 eyes the amplitude was nonrecordable, in 1 eye VEP examination was not performed due to technical issues, and 1 patient had the right eye enucleated after trauma in anamnesis). For 45 of 50 left eyes (LEs) with measurable N1P1 and P1N2 amplitudes, the abnormal N1P1 measures were in 6 of 45 (13 %) and abnormal P1N2 measures were in 5 of 45 (11 %) examined eyes (in a further 5 eyes the amplitude was nonrecordable) (Table 1).

Altogether, 13 of 50 (26 %) patients had abnormal amplitudes at the first examination (including the patients with nonrecordable amplitudes), with unilateral abnormality in 2 of 13 (RE/LE 1/1) and bilateral abnormality in 11 of 13 cases. The correlation was present between the amplitude of evoked complex and the following parameters:

(a) Measured latencies of N1, P1, and N2 peaks of evoked complex ($r = -0.618$, -0.501, and -0.444; $p < 0.001$; 0.001 and 0.003, correspondingly);
(b) Signs of bilateral putaminal necrosis on magnetic resonance imaging (MRI) of brain ($r = 0.420$; $p = 0.004$);
(c) Signs of hemorrhagic brain lesions on MRI of brain ($r = 0.395$; $p = 0.007$);
(d) Serum glucose concentration on admission to hospital ($r = -0.330$; $p = 0.027$);
(e) Serum methanol concentration on admission to hospital ($r = -0.318$; $p = 0.033$).

At the second follow-up examination 24–28 months after acute methanol poisoning, the number of measurements with abnormal and nonrecordable amplitudes had insignificantly increased for N1P1 segment and decreased for P1N2 segment of evoked complex. For 41 REs with measurable both N1P1 and P1N2 amplitudes, N1P1 was abnormal in 5 of 41 (12 %) and P1N2 in 1 of 41 (2 %) cases (in a further 8 eyes the amplitudes were nonrecordable and in 5 eyes it was not measured). For 43 LEs with measurable amplitudes, in 8 of 43 (19 %) cases N1P1 amplitude and in 3 of 43 (7 %) cases P1N2 amplitude were abnormal (in a further 6 eyes the amplitudes were nonrecordable and in 5 eyes it was not measured).

Altogether, 14 of 49 (29 %) patients had abnormal amplitudes on the second examination (including the patients with nonrecordable amplitudes) with unilateral abnormality in 2 of 14 (RE/LE 0/2) and with bilateral abnormality in 12 of 14 of cases. The mean change of amplitude of evoked response during the period between two examinations was insignificant for both N1P1 and P1N2 amplitudes (Fig. 2). The inter-eye differences in the

Table 1 Dynamics of changes of the amplitude of evoked complex during a 2-year period (means with 95 % confidence interval)

	1st Examination ($n = 50$)	% Abnormal	2nd Examination ($n = 49$)	% Abnormal	$P_{1st/2nd}$	Dynamics during 2-year period ($n = 45$)	Difference of inter-eye dynamics ($n = 45$)	$P_{RE/LE}$ dynamics
REs N1P1/μV	6.30 ± 1.10	24	6.50 ± 1.10	27	0.803	0.08 ± 0.59	1.34 ± 0.41	0.337
LEs N1P1/μV	6.56 ± 1.00	22	6.40 ± 1.10	29	0.854	0.49 ± 0.61		
REs P1N2/μV	8.70 ± 1.50	24	9.80 ± 1.60	18	0.331	0.80 ± 1.10	0.08 ± 0.92	0.665
LEs P1N2/μV	8.30 ± 1.40	20	9.30 ± 1.60	18	0.335	0.47 ± 0.96		

REs—right eyes; *LEs*—left eyes; N1P1, P1N2—amplitudes of two major parts of visual evoked potentials; % abnormal—includes abnormal and nonrecordable results, and absent responses; *P*—result of Chi square *t* test

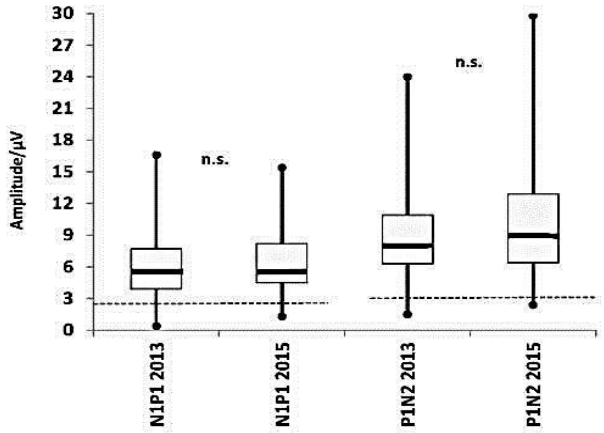

Fig. 2 *Box-and-whisker plot* of dynamics of N1P1 and P1N2 amplitudes change during a 2-year period. The *box* indicates the 25-, 50-, and 75-percentiles, and the points at the ends of the "*whiskers*" are the minimum and the maximum values. *Dotted lines* indicate the low reference limits

dynamics of amplitude changes were not significant as well (all $p > 0.05$).

Of 44 REs with 2 consecutive VEP examinations performed during the study period, in 2 of 44 (5 %) eyes the initially normal N1P1 amplitude became abnormal (decrease from 4.6 to 2.4 μV and from 5.6 to 1.3 μV, correspondingly), in 3 of 44 (7 %) examined eyes the initially abnormal N1P1 and P1N2 amplitudes deteriorated and became nonrecordable, in 7 of 44 (16 %) examined REs the amplitudes remained abnormal and unchanged (including nonrecordable in 4 eyes), and finally in 32 of 44 (73 %) REs the amplitude of both N1P1 and P1N2 segments remained normal without deterioration.

Of 45 LEs with 2 consecutive VEP examinations, in 4 of 45 (9 %) eyes the initially normal N1P1 amplitude (range 3.6–4.9 μV) became abnormal, in 3 of 45 (7 %) eyes the initially abnormal N1P1 and P1N2 amplitudes deteriorated and became nonrecordable, in 7 of 45 (15 %) examined LEs the amplitudes remained abnormal and unchanged (including nonrecordable in 4 eyes), and finally in 31 of 45 (69 %) LEs the amplitude of both N1P1 and P1N2 components remained normal without deterioration.

The correlations were present between the amplitudes of evoked response measured 24–28 month after discharge and the following parameters:

(a) Latencies of N1, P1, and N2 peaks of evoked complex measured on initial examination ($r = -0.681$, -0.514, and -0.425; $p < 0.001$, <0.001, and 0.005, correspondingly);
(b) Latencies of N1, P1, and N2 peaks of evoked complex measured on the second examination ($r = -0.505$, -0.506, and -0.514; all $p < 0.001$);
(c) Amplitudes of N1P1 and P1N2 components of evoked complex measured on initial examination ($r = 0.880$ and 0.839, correspondingly, all $p < 0.001$);
(d) Signs of bilateral putaminal necrosis on MRI of brain ($r = 0.336$; $p = 0.028$);
(e) Serum glucose concentration on admission to hospital ($r = -0.330$; $p = 0.027$);
(f) Serum methanol concentration on admission to hospital ($r = -0.343$; $p = 0.024$).

The negative dynamics of amplitude changes (amplitude deterioration during the 2-year period) correlated with serum lactate ($r = 0.533$; $p < 0.001$), glucose ($r = 0.462$; $p = 0.005$), and formic acid ($r = 0.380$; $p = 0.046$) on admission to hospital. The higher the serum lactate, glucose, and formic acid concentrations, the more pronounce amplitude deterioration occurred in poisoned patients during the study period.

The correlation was present between the prevalence of MR signs of hemorrhagic brain lesions and the amplitude deterioration during the study period ($r = -0.535$; $p < 0.001$). The group consisting of both the patients with abnormal amplitudes of evoked complex measured on initial examination and the patients with amplitude deterioration during the study period had MR signs of hemorrhagic brain lesions more often than those with normal amplitudes without negative dynamics (8/17 vs. 6/33; $p = 0.031$).

Table 2 Laboratory data on admission to hospitals in the patients with acute methanol poisoning (means with 95 % confidence interval)

	S-MetOH/ mmol dm^{-3}	S-Formate[a]/ mmol dm^{-3}	pH	HCO_3^-/ mmol dm^{-3}	*−BD*/ mmol dm^{-3}	*AG*/ mmol dm^{-3}	S-glucose/ mmol dm^{-3}	S-Lactate/ mmol dm^{-3}
Total ($n = 54$)	43.0 ± 13.0	11.3 ± 3.1	7.21 ± 0.06	12.6 ± 2.1	14.6 ± 3.1	26.5 ± 2.9	7.9 ± 1.1	3.3 ± 1.1
Group I ($n = 17$)	62.0 ± 32.0	13.8 ± 7.9	7.06 ± 0.14	9.1 ± 3.9	21.2 ± 6.2	31.7 ± 6.9	10.7 ± 3.0	5.5 ± 2.7
Group II ($n = 33$)	35.0 ± 13.0	10.4 ± 3.6	7.27 ± 0.05	14.1 ± 2.7	11.8 ± 3.4	24.2 ± 3.0	6.7 ± 0.6	2.0 ± 0.5
$P_{I/II}$	0.127	0.367	**0.009**	**0.036**	**0.005**	0.053	**0.015**	**0.018**

Group I—patients with abnormal or nonrecordable amplitudes of evoked complex on the first examination and the patients with amplitude deterioration to abnormal values during the study period in at least one eye; group II—patients with normal amplitudes of evoked complex on the first examination and the patients without amplitude deterioration to abnormal values during the study period; S-MetOH—serum methanol concentration; S-formate—serum formate concentration; HCO_3^-—bicarbonate concentration; *−BD*—base deficit; *AG*—anion gap; S-glucose—serum glucose concentration; S-lactate—serum lactate concentration; *P*—result of Chi square *t* test

Bold values are significant ($p < 0.05$)

[a] Measured in 27 cases

Biochemical and toxicological parameters of acute methanol poisoning and severity of optic nerve axonal degeneration

The key laboratory parameters characterizing the severity of methanol poisoning on admission to hospital for all 54 patients included in the study are presented in Table 2.

The analysis of dynamics of amplitude changes in two groups (group I – the patients with abnormal or nonrecordable amplitudes of evoked complex on initial examination and the patients with amplitude deterioration to abnormal values during the study period in at least one eye ($n = 17$); group II—the patients with normal amplitudes of evoked complex on initial examination and the patients without amplitude deterioration to abnormal values during the study period ($n = 33$) showed prevalently the evidence of deterioration of amplitude of evoked response during the 2-year period in both groups:

(a) In group I (if the amplitudes were recordable on both examinations) the dynamics of N1P1 amplitude changes was negative [REs/LEs mean −0.80 ± 3.10/−1.00 ± 1.60 μV; median 0.10 (range −4.30 to 2.10)/−0.70 (range −2.80 to 0.60) μV], as well as for P1N2 amplitude changes [RE/LE mean −0.1 ± 5.1/−0.3 ± 2 μV; median 0.70 (range −6.30 to 5.30)/−0.20 (range −3.40 to 3.12) μV];

(b) In group II the dynamics of N1P1 amplitude changes was prevalently negative [RE/LE mean 0.02 ± 0.60/−0.32 ± 0.70 μV; median −0.10 (range −4.20 to 2.80)/−0.80 (range −3.40 to 4.80) μV] with slightly positive dynamics for P1N2 amplitude [RE/LE mean 0.90 ± 1.10/0.70 ± 1.10 μV; median 1.60 (range −7.70 to 6.30)/0.70 (range −5.60 to 7.28) μV].

The group I was more severely poisoned on admission to hospitals with lower arterial blood pH, bicarbonate, higher serum methanol, base deficit, and anion gap (all $p < 0.05$). The patients in this group had higher lactacidemia and serum glucose on admission as well (both $p < 0.05$). The patients with higher serum methanol were more severely acidotic: serum methanol concentration correlated with base deficit ($r = -0.362$; $p = 0.009$), arterial blood pH ($r = -0.355$; $p = 0.011$), and anion gap ($r = 0.311$; $p = 0.031$).

The regression analysis of results of the measurements of amplitudes of evoked complex 3–8 months after discharge from hospital showed association of amplitudes with serum glucose and methanol on admission (Fig. 3).

Deterioration of amplitude of evoked complex and biochemical parameters measured at the follow-up examinations

The key laboratory parameters which may potentially affect the dynamics of chronic neuronal degeneration measured during the follow-up period in all 54 patients are presented in Table 3. Four of 54 patients (7 %) had vitamin B_{12} deficiency detected in at least 1 measurement (serum concentration of vitamin B_{12} under 191 ng dm^{-3}): all of them had normal amplitudes of evoked complex at both examinations without negative dynamics during the study period. Nine patients (17 %) had vitamin B_1 deficiency (serum concentration of vitamin B_1 under 42 μg dm^{-3}): seven of them had normal amplitudes of evoked complex and two of them had abnormal evoked complex at initial examination which remained abnormal at the second examination.

Two patients (4 %) had increased serum level of TSH suggesting low production of thyroid hormone. One of them had only a minor increase of serum TSH—5.014 mIU dm^{-3} (normal range 0.5–4.9 mIU dm^{-3}) and normal amplitude of evoked complex. The second patient had 22.69 mIU dm^{-3} serum TSH, but only one VEP

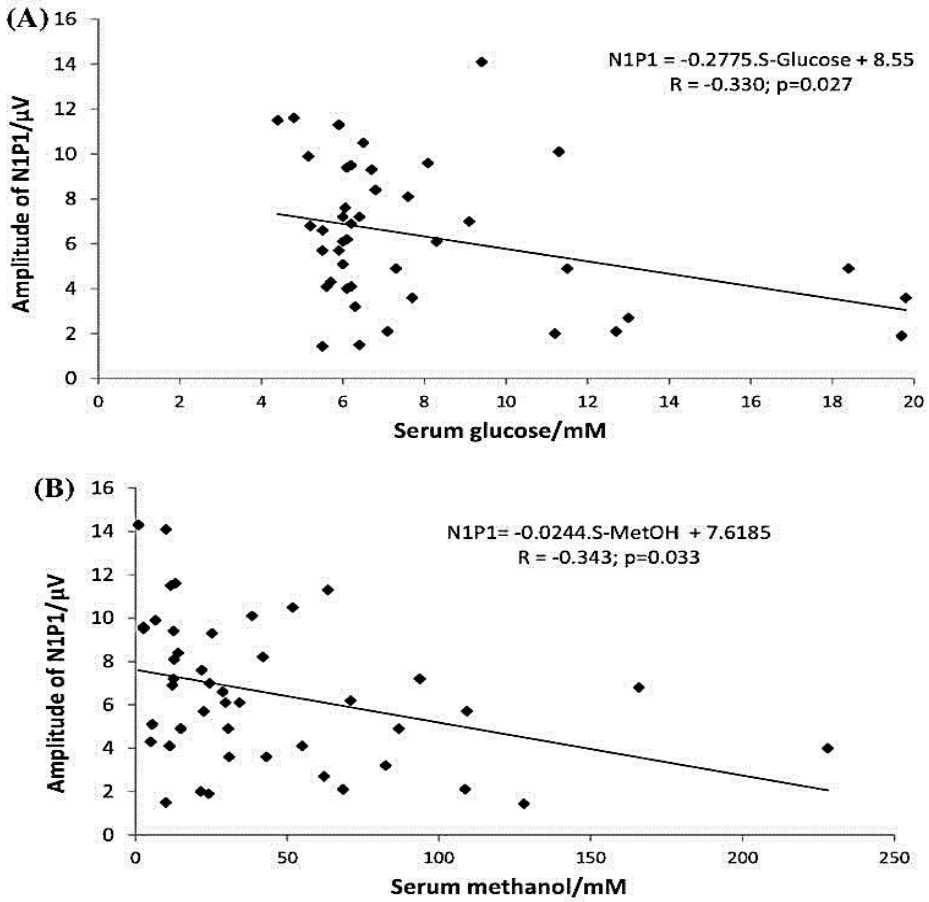

Fig. 3 **a** Serum methanol concentration on admission vs. N1P1 amplitude measured 3–8 months after discharge. **b** Serum glucose concentration on admission vs. N1P1 amplitude measured 3–8 months after discharge

examination performed 2 years after discharge from hospital with normal amplitudes bilaterally.

Of four patients (7 %) with diabetes mellitus, three patients had hyperglycemia poorly controlled during the follow-up period: the first patient with serum glucose of 8.7 and 7.6 mmol dm^{-3} measured during the follow-up study became completely blind during the first 24 h of hospitalization with acute methanol poisoning without any dynamics during the 2-year period; the second patient with serum glucose of 5.7 and 8.5 mmol dm^{-3} had the amplitude nonrecordable in the RE without any dynamics and abnormal in the LE, which remained abnormal at the second examination. Finally, in the third patient with serum glucose of 13.8 and 9.2 mmol dm^{-3}, amplitudes of evoked complex were normal at initial examination and remained normal without negative dynamics during the study period. No correlation of amplitude decrease with CDT, GGT, TSH, serum glucose, vitamin B_{12}, and vitamin B_1 measured at follow-up examinations were present (all $p > 0.05$).

Discussion

The main finding of the study was the functional evidence of neuronal loss in 26 % of the patients 3–8 months after acute methanol-induced optic neuropathy. Further, we found that the process of chronic optic nerve axonal degeneration and neuronal loss can continue over at least two consecutive years in severely poisoned patients. No significant dynamics of amplitude of evoked complex during the 2-year period was observed in most of the cases with initial normal measurements. No patients with initial abnormal amplitudes recovered to normal values 2 years after discharge. On the opposite, in 2 of 44 REs (5 %) and in 4 of 45 LEs (9 %) with 2 consecutive examinations the

Table 3 Biochemical parameters measured on the follow-up examinations (means with 95 % confidence interval)

	CDT (1st)/ %	*CDT* (2nd)/ %	*GGT* (1st)/ ukat dm^{-3}	*GGT* (2nd)/ ukat dm^{-3}	S-Glucose (1st) /mmol dm^{-3}	S-Glucose (2nd)/mmol dm^{-3}	Vitamin B_{12} (1st)/nmol dm^{-3}	Vitamin B_{12} (2nd)/nmol dm^{-3}	Vitamin B_1/nmol dm^{-3}	*TSH* (1st)/ mIU dm^{-3}	*TSH*(2nd)/ mIU dm^{-3}
Total ($n = 54$)	3.0 ± 1.1	2.5 ± 1.1	1.5 ± 0.7	2.1 ± 1.0	5.40 ± 0.47	5.18 ± 0.30	470 ± 170	408 ± 51	59.0 ± 5.1	2.37 ± 0.30	2.74 ± 0.93
Group I ($n = 17$)	2.4 ± 2.1	2.6 ± 2.9	1.2 ± 0.6	2.5 ± 2.0	5.40 ± 0.84	5.21 ± 0.79	720 ± 540	460 ± 120	71.0 ± 12.0	2.28 ± 0.46	2.49 ± 0.66
Group II ($n = 33$)	3.3 ± 1.4	2.7 ± 1.4	1.7 ± 1.0	2.1 ± 1.4	5.40 ± 0.60	5.25 ± 0.35	364 ± 49	385 ± 62	53.8 ± 5.3	2.42 ± 0.40	2.24 ± 0.40
$P_{I/II}$	0.446	0.972	0.366	0.763	1.000	0.916	0.201	0.244	**0.003**	0.661	0.495

Group I—patients with abnormal or nonrecordable amplitudes of evoked complex on the first examination and the patients with amplitude deterioration to abnormal values during the study period in at least one eye; group II—patients with normal amplitudes of evoked complex on the first examination and the patients without amplitude deterioration to abnormal values during the study period; *CDT*—carbohydrate deficient transferrin; *GGT*—gamma-glutamyltransferase; S-Glucose—serum glucose concentration; *TSH*—thyroid-stimulating hormone; 1st—results of first follow-up examination; 2nd—results of second follow-up examination; *P*—result of Chi square *t* test

Bold value is significant ($p < 0.05$)

initially normal amplitudes deteriorated to abnormal values during the study period. In 3 of 45 patients (7 %) the abnormal amplitudes deteriorated in both eyes indicating the ongoing process of chronic neuronal degeneration.

The degree of initial axonal damage positively correlated with serum methanol and glucose concentrations on admission to hospital. The dynamics of chronic degenerative changes was associated with the degree of acidosis and severity of poisoning characterized by serum lactate, formic acid, and glucose concentrations on admission to hospital. The amplitude of evoked complex correlated with brain MR findings: the abnormal amplitudes or nonrecordable responses were present mainly in the patients with bilateral putaminal necrosis. The negative dynamics of amplitude (deterioration) during 2 years of study was associated with the presence of MR signs of hemorrhagic lesions in the brain.

Formic acid as the major toxic metabolite of methanol in humans has both myelinoclastic and direct toxic effects on axons of the optic nerve [12]. The interaction and interdependency of histotoxic effect with demyelinating and axonal-degenerative components in methanol-induced optic neuropathy is not clearly understood. During acute poisoning, swelling of damaged myelin sheaths leads to a compression-type injury to the nerve fibers, conduction deficit and possible complete block [13, 26]. Acute transection of axons along the anterior visual pathway leads to retrograde degeneration, which ultimately reaches the retinal ganglion cells. The studies on acute optic neuritis in the patients with multiple sclerosis indicated that massive axonal injury may occur during the first weeks after the onset of demyelination [27].

Axonal degeneration and retinal nerve fiber layer (RNFL) thinning 2–3 months after an acute episode of demyelination have been described in multiple sclerosis, neuromyelitis optica, and idiopathic optic neuritis [28, 29]. The RNFL values continue to decrease for at least 6–12 months after the symptoms onset [30]. Extensive and persistent decrease of the amplitude of evoked complex may occur after single episode of acute optic neuritis with possible deterioration during at least 1 year after the attack [31]. Thin axons subserving the central visual field are more susceptible to damage than thick axons from the periphery [32].

Conduction block caused by myelin sheath swelling and axonal compression by edema normally recovers within few weeks, and the degree of decrease of the amplitude of evoked complex detected later than 2 months after acute methanol poisoning is the manifestation of the extent of neuronal loss mainly in the central visual field. In our study, abnormal amplitudes measured 3–8 months after discharge from hospital were present in 26 % of patients suggesting high prevalence of acute axonal loss in methanol optic neuropathy. The direct relationship exists between the extent of acute demyelination and consequent axonal loss in optic neuritis [25]. In our study, strong negative correlation was present between the measured P1 latencies characterizing the extent of myelin sheaths damage and the amplitudes of evoked complex, which confirmed the suggestion of Klistoner et al. (2008) on the role of demyelination in promoting axonal loss [25].

The patients with abnormal amplitudes had significantly higher serum concentrations of methanol and glucose on admission than those with normal amplitudes. In earlier studies, it was shown that serum methanol concentration had no independent prognostic value, but patients with higher serum methanol were commonly more severely poisoned [4, 6, 20]. In our study, serum methanol concentration correlated with the degree of metabolic acidosis, explaining the positive association between this

toxicological parameter and the amplitude decrease. Stress-induced hyperglycemia could mean deeper and longer hypoxia and more serious damage of both neurons and oligodendrocytes. High serum glucose seen in critically ill patients is also a known prognostic factor of poor outcome [33].

Strong association exists between structural and functional measures of the optic nerve integrity [25]. We found that functional measures of the optic nerve axonal damage may provide important screening information on brain damage in methanol poisoned patients. The association was present between the abnormal amplitudes of evoked complex and the signs of bilateral putaminal necrosis and hemorrhagic brain lesions on MR examination (both $p < 0.01$). This association suggests that the same biochemical and toxicological determinants may play a role in the prevalence and magnitude of central nervous system damage in acute methanol poisonings.

The dynamics of possible changes of amplitude of evoked response during the years following acute optic neuropathy was not studied. In the study of Klistorner et al. (2010), there was a significant restoration of amplitude between 6 and 12 months of follow-up after acute unilateral optic neuritis in 25 subjects without previous demyelinating events, implying continuous recovery of amplitude despite the loss of RNFL fibers [34]. The amplitude improved by 17.8 %, whereas RNFL thickness worsened by 20.8 %. The authors suggested that continuous cortical reorganization (neuroplasticity) or remodeling of function within the visual system in postacute phase might play a role in long-term functional improvement and was responsible for the increase in amplitude.

In our study, we did not find the evidence of significant restoration of amplitude of evoked complex during a 2-year period. The total number of abnormal measurements increased; new cases with abnormal amplitudes were detected and deterioration of abnormal amplitudes was observed, whereas no cases with the amplitude restoration from abnormal to normal values were registered. In the patients with initially abnormal amplitudes of evoked complex the dynamics was negative with a further deterioration to more pronounced decrease or even to nonrecordable amplitudes. In the group of 37 patients with normal amplitude of evoked complex at initial examination, the results of measurements in four (11 %) patients indicated deterioration to abnormal values; the median of N1P1 amplitude dynamics was negative for both eyes. Although the results of the present study cannot be directly compared with the studies of amplitude in optic neuritis, the data suggest that the dynamics of amplitude changes after acute methanol-induced optic neuropathy is prevalently negative for at least 24–28 months. Further studies are necessary to find out if the amplitude deterioration reaches plateau or continues during the following years.

The dynamics of amplitude deterioration demonstrated the association with degree of metabolic acidosis (serum concentration of lactate and formic acid) and glycemia on admission, the parameters characterizing severity and duration of cell hypoxia in acute methanol poisoning. It is known that exposure of neurons to hypoxia induces broad range of biochemical and genetic mechanisms leading to apoptotic cell death and the severity of hypoxic insult influences the pattern of pro-apoptotic genes expression [35]. Therefore, the mechanisms of chronic neuronal degeneration underlying the amplitude deterioration of evoked complex may be related to the activation of a genetic program of apoptotic neuronal death following formic acid-induced cellular hypoxia. This fact raises the question whether inhibition of neuronal apoptosis following hypoxia will contribute to improved visual outcome and what pharmacological interventions may be potentially helpful in interrupting the apoptotic mechanisms in optic neurons surviving acute damage.

Several conditions like vitamin B_{12} or B_1 deficiency caused by chronic alcoholism or nutritional deficits, thyroid gland hypofunction, or poorly controlled glycemia in diabetes mellitus may negatively affect the mechanism of remyelination during the period following acute methanol-induced optic neuropathy and cause amplitude deterioration due to inadequate restoration of myelin sheaths trophic and protective functions [36–42]. In our study, no association was found between the concentration of serum glucose, TSH, vitamin B_{12}, and vitamin B_1 measured at both follow-up examinations and dynamics of amplitude of evoked complex during a 2-year period. The reason for the absence of association might be the small number of patients with diabetes mellitus, hypothyroidism, and hypovitaminosis.

Strength and limitations

The study has some principal limitations, the most important one being the lack of VEP measurement before acute methanol poisoning, during treatment in hospitals, and on discharge from hospital, which prevents the comparison of amplitudes measured during the follow-up period with the amplitudes of the "intact" optic nerve before toxic exposure and the amplitudes at the peak of toxic exposure. The time between discharge from hospital and the follow-up VEP measurements differed in the 54 patients from 3 to 8 months for the first examination and from 24 to 28 months for the second examination, making possible additional inter-individual variability in the results of measurements. The study was not controlled relating to treatment modalities (choice of antidote and mode of

dialysis, alkalinization, and folate substitution) which could affect the outcome of methanol poisonings. A substantial number of the survivors of the acute poisonings chose not to participate in the follow-up study; therefore, the selection bias might be present with prevalently less severely affected patients participating in the follow-up examinations.

Despite the limitations and confounders, this is the first prospective study performed after a mass methanol poisoning outbreak comparing the dynamics of amplitude of evoked complex in surviving patients in association with key toxicological and biochemical parameters measured on admission to hospital and during a 2-year follow-up period, representing the most comprehensive data ever generated in the field. The essential clinical and laboratory data were collected during the Czech mass outbreak in a prospective manner using standardized forms; the follow-up examinations were performed in one medical facility using a uniform investigation protocol.

Methods

Patients

The study was designed as a prospective cross-sectional examiner-masked study. During the Czech mass methanol outbreak, all cases of confirmed methanol poisonings treated in hospitals were documented using a standardized admission protocol, and discharge reports of all hospitalized patients with confirmed diagnosis and results of neurological and ophthalmologic examinations on admission, during hospitalization, and on discharge were collected and analyzed in the Czech Toxicological Information Center.

The inclusion criteria for the study were: (a) confirmed diagnosis of acute methanol poisoning (see below); (b) survival of methanol poisoning and discharge from hospital; (c) written informed consent with clinical examination according to the study protocol. The exclusion criteria were: (a) the presence of multiple sclerosis and other demyelinating and neurodegenerative diseases; (b) the patients examined only once during the study period were excluded from the analysis of dynamics of amplitude of evoked complex.

On admission, the laboratory investigations included serum concentrations of methanol, ethanol, formate, lactate, electrolytes, arterial blood gases, anion and osmolal gaps, glucose, urea, creatinine, bilirubin, liver enzymes, complete blood count, hematocrit, and serum proteins. The diagnosis was established if: (1) a history of recent ingestion of illicit spirit was available, and serum methanol concentration was more than 6.2 mmol dm^{-3} (200 mg dm^{-3}), or (2) there was a history/clinical suspicion of methanol poisoning, serum methanol detectable, and at least two of the following were present: pH less than 7.3, serum bicarbonate less 20 mmol dm^{-3} (20 mEq dm^{-3}), and anion gap more than 19 mmol dm^{-3} (19 mEq dm^{-3}). The ophthalmologic examinations during hospitalization and on discharge included fundus examination and standard ophthalmic tests.

The patients were treated in accordance with the American Academy of Clinical Toxicology (AACT) and European Association of Poisons Centres and Clinical Toxicologists (EAPCCT) practice guidelines for the treatment of methanol poisoning [12]. Bicarbonate was given as a buffer to the patients with metabolic acidosis. Ethanol or fomepizole was used as antidote to block alcohol dehydrogenase enzyme [19, 43]. Intermittent hemodialysis (IHD) or continuous veno-venous hemodialysis/hemodiafiltration (CVVHD/HDF) was performed to eliminate formic acid and methanol and correct the metabolic acidosis [44]. Folates were administered in 63 patients: folic acid in 35, and folinic acid in 28 subjects [16]. Corticosteroids were not administered to the patients with visual disturbances.

Laboratory investigations

Methanol was measured by a gas chromatographic method with flame ionization detection and a direct injection with internal standard (Gas Chromatograph Chrom 5, Laboratory Instruments Prague, Czech Republic), limit of detection 60 mg dm^{-3} (1.9 mmol dm^{-3}) and day-to-day coefficient of variation 2.5–5.4 % [45]. Calibrators and controls were made by dilution of methanol p.a. (Penta, Czech Republic). Formate was measured enzymatically on a Hitachi analyzer (Hitachi 912, Hitachi Science Systems Ltd., Japan) using formate dehydrogenase (Roche, France) and nicotinamide adenine dinucleotide (NAD) (Roche, France). Pure sodium formate (Sigma-Aldrich, USA) was used to prepare a standard of 46 mg dm^{-3} (1.0 mmol dm^{-3}) in phosphate buffer and two control sera. Day-to-day coefficient of variation was 5.6 %, and the upper reference limit was 2 mg dm^{-3} (0.44 mmol dm^{-3}). Serum ethanol was analyzed by gas chromatography with flame ionization detection and direct injection with an internal standard (Gas Chromatograph Chrom 5, Laboratory Instruments Prague, Czech Republic). The limit of detection was 40 mg dm^{-3} (0.87 mmol dm^{-3}), and the day-to-day coefficient of variation was 3.8 to 7.1 % [46]. Ethanol standards were purchased (Erba Lachema, Czech Republic).

The anion gap in serum was determined from the equation:

$$AG = (Na^+ + K^+) - (Cl^- + HCO_3^-)$$

where AG—anion gap, Na^+—serum concentration of sodium, K^+—serum concentration of potassium, Cl^-—

serum concentration of chlorine, HCO_3^-—serum concentration of bicarbonate. The reference range is 13 + 8 mmol dm^{-3} (mean + 2SD) [47].

Clinical investigation protocol

The clinical examination protocol 3–8 months (first examination) and 24–28 months (second examination) after discharge from hospitals included complete ocular examination and standard ophthalmic tests (visual acuity, color vision, contrast sensitivity, perimeter, and fundoscopy), full-field visual evoked potentials (VEP), biochemical tests (electrolytes, glucose, glycohemoglobin, albumin, pre-albumin, urea, creatinine, bilirubin, liver enzymes, cholesterol, lipids, thyroid-stimulating hormone (TSH), vitamin B_{12}, vitamin B_1, and carbohydrate-deficient transferrin), qualitative ethyl glucuronide in urine), and standardized questionnaire forms. The examiners were masked to the serum methanol and formic acid concentrations on admission, severity of poisoning, clinical course, treatment measures and outcomes in methanol-poisoned patients on discharge from hospitals, as well as to each other's results.

VEP examination was performed on two-channel device TruTrace 4 Alien Technik CZ. Monocular checkerboard pattern-reversal stimulation was used, with frequency of 1.5 c s^{-1}, angular size of the monitor 6° × 5° from the fixation point, angular size of checkerboard squares 40′. Luminance of the white and black squares was 84 cd m^{-2} and 57 cd m^{-2}, respectively. Bandwidth of the amplifier was 1 Hz–1 kHz, the evoked response was registered from the Oz-Fz derivation. At each eye, the examination was performed twice in order to check reproducibility of the evoked complex. We evaluated the amplitudes of N1P1 and P1N2 components of the evoked complex. The measured values in patients were compared with our laboratory reference values determined as the central 95 % interquartile interval of data measured on a group of 30 healthy individuals. Three criteria of latency abnormality were chosen: (1) amplitude of N1P1 part of evoked complex <2.6 μV, (2) amplitude of P1N2 part of evoked complex <3.0 μV, and (3) absence of response. The result was categorized as abnormal if at least one of the above-mentioned criteria was fulfilled.

The patients underwent two follow-up MRI examinations 3–8 months and 24–28 months after discharge from hospitals on Gyroscan Phillips, 1.5 T with the same protocol: axial *T*2-weighted image with slice thickness (THK) 6.0/0.6 mm through the whole brain, with parameters: repetition time (*TR*) 4241 ms, time to echo (*TE*) 100 ms, flip angle (*FA*) 90°, FLAIR (fluid attenuated inversion recovery): *TR* 11,000 ms, *TE* 140 ms, inversion time (*TI*) 2800 ms, *FA* 90°, *T*1-weighted image: *TR* 569 ms, *TE* 15 ms, *FA* 69°, *T*2-weighted image—fast field echo: *TR* 665 ms, *TE* 23 ms, *FA* 18°, single shot diffusion-weighted image: *TR* 2901 ms, *TE* 75 ms, *FA* 90°, *T*1-weighted after administration of contrast medium (Gd) and in coronal images centered to the orbital region *T*2-weighted image with fat suppression (SPIR): *TR* 5506 ms, *TE* 100 ms, *FA* 90°. The patients were considered as having CNS sequelae of poisoning if symmetrical necrosis, with or without hemorrhages, of basal ganglia (putamen, globus pallidum) and other brain lesions (brainstem, nucleus caudatum, cerebellum, deposits in white matter, and optical nerve atrophy) compatible with the diagnosis of acute methanol poisoning were present on MR scan of the brain.

Statistical analyses

The admission laboratory data in the different groups were compared on a group by group basis using two-sample *t* test assuming unequal variances (equal means), two-sample *F* test for variances, bias test, and two-sample Kolmogorov–Smirnov test. Data are expressed as arithmetic means with confidence intervals. For comparison of the obtained results, common statistical tests have been used (*t* test: two-sample assuming equal variances, *t* test: two-sample assuming unequal variances (equal means), two-sample *F* test for variances, bias test, and ANOVA). Chi square tests were used to examine the differences between the groups with normal and abnormal amplitudes of evoked complex. Pearson's correlation and linear regression analysis were used to examine the relationships between various parameters. All statistical calculations were carried out with a level of significance $\alpha = 0.05$. Statistical documentation was performed in Excel (Microsoft, USA), and the formal calculations were produced in QC Expert software 3.1 (Trilobyte, Pardubice, Czech Republic) and in IBM SPSS ver. 17.0 and Statistica SF ver. 10.0 (both licensed to 1st Faculty of Medicine of Charles University in Prague).

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Compliance with ethical standards

Ethics The study was approved by the General University Hospital Ethics Committee in Prague, Czech Republic. Informed consent was obtained from the participants of the study. The study adhered to the tenets of the Declaration of Helsinki.

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ORIGINAL PAPER

Prevalence, dynamics, and biochemical predictors of optic nerve remyelination after methanol-induced acute optic neuropathy: a 2-year prospective study in 54 patients

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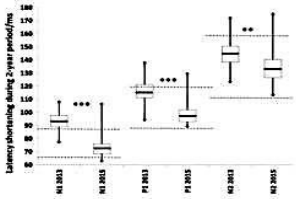
Abstract We conducted a prospective study in 54 patients with a median age of 48 years (range 23–73) to determine the prevalence and dynamics of optic nerve remyelination after methanol-induced optic neuropathy. Methanol was measured by a gas chromatographic method with flame ionization detection. Formate was measured enzymatically. Measurement of full-field visual evoked potential with monocular checkerboard pattern-reversal stimulation was performed 3–8 and 24–28 months after discharge. The latency of the positive peak (P1) was used for the analysis of remyelination dynamics. Twenty-seven patients had abnormal P1 latencies. Mean P1 latency for right eyes (REs) was 115.3 ± 2.1 ms and for left eyes (LEs) was 117.4 ± 3.2 ms. The group with abnormal latency had lower arterial pH ($p = 0.017$), higher anion gap ($p = 0.013$), methanol ($p = 0.027$), base deficit ($p = 0.033$), and lactate ($p = 0.048$). At the second examination, shortening of P1 latencies was registered (REs/LEs 98.3 ± 2.4/102.7 ± 4.5 ms; $p < 0.001$). The dynamics of latency shortening for REs/LEs were 17 ± 1.3/15.1 ± 3.1 ms, with insignificant inter-eye difference ($p = 0.271$). The dynamics of remyelination correlated with serum methanol ($r = -0.588$; $p < 0.001$), arterial pH ($r = 0.339$; $p = 0.040$), and concentration of carbohydrate-deficient transferrin ($r = -0.411$; $p = 0.011$). Remyelination occurred in cases of mild or moderate damage of myelin sheaths; no improvement of conduction was found in severe cases. The dynamics of remyelination correlated with the degree of acidosis and severity of poisoning. Chronic alcohol abuse had a negative effect on remyelination dynamics.

Graphical abstract



Keywords Methanol optic neuropathy · Visual evoked potentials · Remyelination · Visual sequelae of poisoning · Prognosis · Metabolic acidosis

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Introduction

Background

Outbreaks of mass or cluster methanol poisonings due to consumption of illicit alcohol occur frequently and remain a serious problem for healthcare providers due to high morbidity and mortality [1–5]. More than 50 mass methanol outbreaks with about 5000 poisoned subjects and

more than 2000 fatalities occurred worldwide during 2000–2012 [6]. If medical treatment is inadequate or delayed, mortality exceeding 40 % and severe long-term visual impairment may occur [7].

Methanol is metabolized in humans to the highly toxic formic acid/formate anion, which inhibits mitochondrial respiration [8, 9]. The accumulation of formic acid leads to metabolic acidosis and may result in optic neuropathy, and damage of the retina and the basal ganglia when its concentration rises above 9–10 mmol dm^{-3} [10–13]. Prevention of methanol metabolism by blocking the alcohol dehydrogenase enzyme (ADH) with antidotes (fomepizole or ethanol) and correction of metabolic acidosis with bicarbonate and dialysis are needed for successful treatment [14–16].

The axons of retinal ganglion cells, which form the optic nerve, are selectively vulnerable to histotoxic hypoxia caused by formic acid because of their high energy dependence [17, 18]. The symptoms of ocular toxicity manifest after a latency period of 8–48 or more hours, depending on the amount of methanol ingested, possible ethanol co-ingestion, and other factors. In many cases, a complete recovery with resolution of changes of the fundus, improvement of visual acuity, and extinction of subjective symptoms occurs within 4–8 weeks. However, long-term visual damage may persist in 10–30 % of poisoned patients [19, 20]. Long-term visual sequelae may include peripheral constriction of visual fields and/or central scotoma, reduced visual acuity, loss of color vision, and finally, complete blindness in the most serious cases.

Importance

The known biochemical and toxicological parameters predicting mortality in acute methanol poisonings are severity of metabolic acidosis (low arterial blood pH and bicarbonate, high anion gap and base deficit), high serum lactate and formate concentrations, hyperglycemia, and negative serum ethanol on admission [21–23]. In survivors of methanol poisoning, however, the effects of these determinants on the prevalence and dynamics of possible optic nerve recovery during the years following the acute toxic neuropathy have not been studied.

Visual evoked potential (VEP) measurements can provide information about the dynamics of optic nerve conductivity changes associated with myelin repair and restoration of the integrity of the visual pathway. The latency of full-field VEPs reflects the degree of demyelination of the optic nerve fibers (the extent of the demyelinated area) and subsequent shortening of latency represents the ongoing process of remyelination [24].

Acute demyelination caused by formic acid in methanol-induced optic neuropathy can lead to axonal degeneration due to the lack of trophic support from myelin and the disruption of normal axon–myelin interaction. From electrophysiological studies of acute optic neuritis in patients with multiple sclerosis it is known that the process of spontaneous remyelination may occur during the first 2–3 years after the acute episode [25]. Latency reduction of 6–7 ms was observed at follow-up VEP examinations during the first 6 months and of a further 4 ms between 6 months and 2 years after acute optic neuritis in patients in the study of Brusa et al. [26].

Remyelination of the optic nerve after acute damage is an important factor for the prevention of chronic axonal loss, retrograde retinal nerve ganglion cell death, and further long-term deterioration of visual functions after acute methanol poisoning. There are data from clinical case reports about the possibility of recovery of visual functions within several months following the poisoning [27]. On the other hand, the results of a follow-up study by Paasma et al. [19] in Estonia suggest the possibility of new—or unrecognized—visual complications in up to 36 % of patients during the 6 years following acute poisoning. Therefore, knowledge of biochemical predictors associated with the dynamics of remyelination of the optic nerve and objective measures of this process are critical in both the prediction of the character of long-term visual sequelae of acute methanol poisoning and in the assessment of the clinical effectiveness of therapeutic interventions (folate substitution, different modes of hemodialysis, type of antidote, and others).

Goals of this investigation

In this study we aimed to examine the prevalence and dynamics of optic nerve remyelination and to study the association between biochemical and toxicological parameters of acute methanol poisoning measured in blood serum on admission to hospital and at the follow-up clinical examination and the functional measures of optic nerve integrity during a 2-year period in a cohort of patients with confirmed acute methanol poisoning. Our hypothesis was that the dynamics of remyelination after methanol-induced optic neuropathy would be associated with certain biochemical and toxicological parameters of poisoning (degree of metabolic acidosis, concentrations of toxic agent and its metabolite, and others).

Results and discussion

Demographic characteristics

During the Czech methanol outbreak from September 2012 to August 2014, 137 patients were poisoned and 106 of them were treated in hospital; 83 patients survived

methanol poisoning. A modified protocol for collection of anamnestic, clinical, toxicological, and biochemical laboratory admission data based on experience from a methanol outbreak in Norway in 2002–2004 [1] was distributed to all hospitals during the second week of the outbreak and used for the prospective data collection. A detailed history of the poisoning, and of the onset and dynamics of ocular and systemic toxicity, was obtained in a prospective manner directly from the patients or from relatives of critically ill patients upon admission to the secondary hospital. The data for the patients admitted before distribution of the protocol were collected retrospectively. Direct communication by phone and email with medical doctors who admitted and treated poisoned patients, as well as with the heads of emergency departments, in 30 hospitals in 11 regions of the Czech Republic was undertaken to clarify the key data, if necessary. The discharge reports of all hospitalized patients with a confirmed diagnosis and the results of computer tomography (CT), magnetic resonance imaging (MRI), neurological and ophthalmological examinations on admission, during hospitalization, and on discharge were collected retrospectively and analyzed in the Toxicological Information Center (TIC).

All 83 patients who survived acute methanol poisoning were invited to participate in a 1-day outpatient clinical examination twice during the study period: 3–8 and 24–28 months after discharge from hospital. Fifty four patients (65 %) with a median age of 48 years (range 23–73 years), 45 males and 9 females, agreed and were subjected to the follow-up clinical examinations. Of them, five patients only participated in the first clinical examination 3–8 months after poisoning (three of these patients died 1–2 years after acute methanol poisoning, one patient rejected further participation in the study, and contact was lost with the fifth patient). Further, four patients agreed to participate in the study after the first follow-up clinical examination of the population had already been performed and were examined only once, during the second follow-up examination. The results of latency measurements in these nine patients were excluded from the analysis of association of the dynamics of P1 latency changes during a 2-year period with biochemical and toxicological parameters.

The median ingested volume of toxic spirits was 300 cm^3 (range 100–1500 cm^3) comprising approximately 50 % methanol and 50 % ethanol in different kinds of strong alcoholic beverage with a total alcohol content of around 40 % alcohol by volume (ABV, v/v). Only 22 % of the patients were diagnosed within 12 h of methanol ingestion, 66 % within 48 h, and 6 % later than 48 h (in 6 % it was impossible to determine the time of ingestion reliably). A medical history of chronic alcohol abuse was present in 48 % of the cases. Regarding somatic diseases, 21 patients had hyperlipidemia, 19 patients had arterial hypertension, 16 patients had alcoholic hepatopathy, 4 patients had diabetes mellitus (type II in 3 cases, type I in 1 case), 3 patients had myocardial infarction, and 1 patient had had brain stroke in anamnesis before methanol poisoning; epilepsy, sarcoidosis, psoriasis vulgaris, chronic atrial fibrillation, and ischemic disease of the lower extremities were present each in 1 patient. No patients with multiple sclerosis and other demyelinating and neurodegenerative diseases were present in the study population.

The dynamics of latency changes of major components of VEP in the 2 years following poisoning

On initial examination at 3–8 months after discharge from hospital, P1 latencies were measurable for 42 of 50 right eyes (REs) and were abnormally prolonged in 16 of 42 (38 %) of them (in a further 6 eyes the P1 latency was nonrecordable, in 1 eye VEP was not measured due to technical issues, and 1 patient had the right eye enucleated after trauma in anamnesis). For 44 of 50 left eyes (LEs) with measurable P1 latencies, the abnormal measures were in 20 of 44 (45 %) examined LEs (in a further 6 eyes the P1 latency was nonrecordable) (Table 1).

Altogether, 27 of 50 (54 %) patients had abnormal P1 latencies at the first examination (including the patients with nonrecordable P1 latency), with unilateral abnormality in 6 of 27 (REs/LEs 2/4) and bilateral abnormality in 21 of 27 cases. The correlation was present between P1 latencies and the following biochemical parameters measured in methanol poisoned patients on admission to hospital (Table 2):

(a) anion gap ($r = 0.390$; $p = 0.010$),
(b) arterial blood pH ($r = -0.338$; $p = 0.029$),
(c) serum methanol ($r = 0.312$; $p = 0.037$),
(d) serum glucose ($r = 0.301$; $p = 0.045$).

At the second follow-up examination 24–28 months after acute methanol poisoning, the number of abnormal P1 latencies of evoked responses had significantly decreased. For 41 REs with measurable P1 latencies, only 1 of 41 (2 %) was abnormal (in a further 8 eyes the P1 latencies were nonrecordable and in 5 eyes it was not measured). For 43 LEs with measurable P1 latencies, in 5 of 43 (12 %) it was abnormal (in further 6 eyes the P1 latency was nonrecordable and in 5 eyes it was not measured).

The latency shortening of the evoked response was significant for all three measured peaks with the maximal registered dynamics of N1 latency shortening (Fig. 1). The inter-eye differences in the dynamics of latency shortening during the study period were not significant for all three measured peaks (N1, P1, and N2).

Of 44 REs with two consecutive VEP examinations performed during the study period, in 3 of 44 (7 %) eyes

Table 1 Dynamics of changes of latency of major components of the evoked potentials obtained from a group of 54 patients during a 2-year period (means with 95 % confidence interval)

	1st examination	% abnormal	2nd examination	% abnormal	$P_{1st/2nd}$	Dynamics during 2-year period	Difference of inter-eye dynamics	$P_{RE/LE}$ dynamics
REs N1/ms	92.7 ± 1.8	81	72.7 ± 2.3	2	<0.001	20.2 ± 2.1	2.1 ± 4.2	0.455
LEs N1/ms	94.2 ± 2.6	73	75.5 ± 4.6	9	<0.001	18.5 ± 4.3		
REs P1/ms	115.3 ± 2.1	38	98.3 ± 2.4	2	<0.001	17 ± 1.3	2.0 ± 3.2	0.271
LEs P1/ms	117.4 ± 3.2	45	102.7 ± 4.5	12	<0.001	15.1 ± 3.1		
REs N2/ms	144.0 ± 2.9	5	134.1 ± 3.7	0	<0.001	9.4 ± 2.9	2.6 ± 3.8	0.250
LEs N2/ms	145.7 ± 3.7	11	138.7 ± 5.6	12	0.042	6.5 ± 4.0		

REs, right eyes; LEs, left eyes; N1, P1, N2, latencies of three major peaks (two negative and one positive) of visual evoked potentials; *P*, result of Chi square *t* test

Table 2 Laboratory data on admission in 54 patients with acute methanol poisoning (means with 95 % confidence interval)

	S-MetOH/ mmol dm^{-3}	S-formate[a]/ mmol dm^{-3}	pH	HCO_3/ mmol dm^{-3}	−BD/ mmol dm^{-3}	AG/ mmol dm^{-3}	S-glucose/ mmol dm^{-3}	S-lactate/ mmol dm^{-3}
Total ($n = 54$)	43.0 ± 13.0	11.3 ± 3.1	7.21 ± 0.06	12.6 ± 2.1	14.6 ± 3.1	26.5 ± 2.9	7.9 ± 1.1	3.3 ± 1.1
Group I ($n = 27$)	57 ± 23	11.4 ± 4.4	7.13 ± 0.10	10.5 ± 3.0	18.2 ± 4.6	30.3 ± 4.3	9.1 ± 2.1	4.3 ± 2.0
Group II ($n = 23$)	29 ± 11	11.1 ± 5.0	7.274 ± 0.069	14.5 ± 3.4	11.3 ± 4.4	22.6 ± 4.0	6.94 ± 0.81	2.20 ± 0.63
$P_{I/II}$	**0.027**	0.909	**0.017**	0.075	**0.033**	**0.013**	0.062	**0.048**

Group I, patients with abnormal P1 latency on first follow-up examination on at least one eye (including the patients with P1 latency not measurable and the absent response); Group II, patients with normal P1 latency measured on first follow-up examination; S-MetOH, serum methanol concentration; S-formate, serum formate concentration; HCO_3^-, bicarbonate concentration; −BD, base deficit; AG, anion gap; S-glucose, serum glucose concentration; S-lactate, serum lactate concentration; *P*, result of Chi square *t* test

Significant values are highlighted in bold ($p < 0.05$)

[a] Measured in 27 cases

the initially abnormally prolonged P1 latency became nonrecordable (worsening of VEP findings), in 4 of 44 (9 %) eyes the P1 latency remained unchanged (nonrecordable), and in 37 of 44 (84 %) the latency of P1 shortened. After the elimination of three REs with measurable P1 latency at initial examination, which became nonrecordable at the second examination, the latency shortening during a 2-year period remained significant (114.8 ± 2.3 vs. 98.39 ± 2.4 ms, $p < 0.001$).

Of 45 LEs with 2 consecutive VEP examinations, in 4 of 45 (9 %) eyes the initially abnormally prolonged P1 latency became more abnormal (nonrecordable in 3 eyes and increased P1 latency in 1 eye), in 2 of 45 (4 %) eyes the P1 latency remained unchanged (nonrecordable), and in 39 of 45 (87 %) the latency of P1 shortened. No improvement of conductivity occurred over the 24–28 months after discharge in the patients with initial severe damage detected at the first follow-up examination (patients with P1 latency nonrecordable). After the elimination of three LEs with measurable P1 latency at initial examination, which became nonrecordable at the second examination, the latency shortening during a 2-year period remained significant (116.6 ± 3.3 vs. 102.7 ± 4.5 ms, $p < 0.001$).

The correlations were present between P1 latencies 24–28 months after acute poisoning and the following biochemical parameters measured in methanol poisoned patients on admission to hospital:

(a) serum methanol ($r = 0.505$; $p = 0.001$),
(b) arterial blood pH ($r = -0.373$; $p = 0.014$),
(c) base deficit ($r = -0.339$; $p = 0.026$).

The dynamics of P1 latency shortening correlated with serum methanol concentration ($r = -0.588$; $p < 0.001$) and arterial blood pH ($r = 0.339$; $p = 0.040$) on admission. The higher the serum methanol concentration and lower the arterial blood pH (more severe metabolic acidosis) in poisoned patients, the lower the dynamics of P1 latency shortening during the study period.

Biochemical and toxicological parameters of acute methanol poisoning and severity of optic nerve demyelination

The key laboratory parameters characterizing severity of methanol poisoning on admission to hospitals for all 54 patients included in the study are presented in Table 2. The

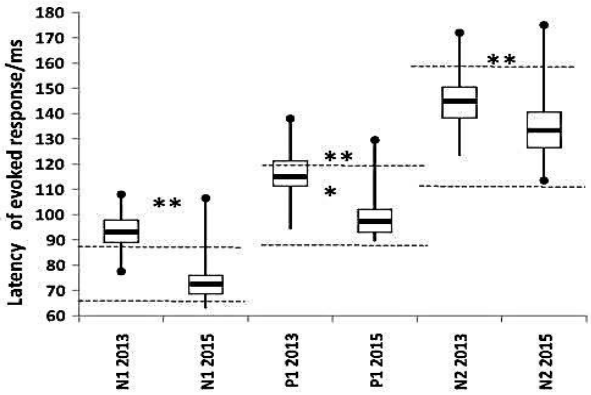

Fig. 1 *Box*-and-*whisker plot* of dynamics of latency shortening of N1, P1, and N2 peaks of evoked responses during 2-year period. The *box* indicates the 25-, 50-, and 75-percentiles, and the points at the ends of the "*whiskers*" are the minimum and the maximum values. *Dotted lines* indicate the reference limits. $***p < 0.001$; $**p < 0.01$

group of patients with abnormal or nonrecordable P1 latencies at the first examination was more severely poisoned, with lower arterial blood pH, higher serum methanol, base deficit, anion gap, and lactate (all $p < 0.050$). The patients in this group had insignificantly higher serum glucose on admission ($p = 0.062$). The patients with higher serum methanol were more severely acidotic: serum methanol concentration correlated with base deficit ($r = -0.362$; $p = 0.009$), arterial blood pH ($r = -0.355$; $p = 0.011$), and anion gap ($r = 0.311$; $p = 0.031$).

The regression analysis of results of the measurement of P1 latencies 3–8 months after discharge from hospital showed association of P1 latency prolongation with anion gap, arterial blood pH, serum methanol and glucose on admission (Fig. 2).

The dynamics of P1 latency shortening during the study period depended on the severity of poisoning as well. Figure 3 presents the association between the dynamics of P1 latency shortening and arterial blood pH and serum methanol concentration on admission (Fig. 3).

Dynamics of optic nerve remyelination and biochemical parameters measured at the follow-up examinations

The key laboratory parameters which may potentially affect the dynamics of remyelination of the optic nerve, measured during the follow-up period in all 54 patients included in the study are presented in Table 3. Four of 54 patients (7 %) had vitamin B_{12} deficiency detected in at least one measurement (serum concentration of vitamin B_{12} under 191 ng dm^{-3}): 2 of them had abnormal P1 latencies at the first examination (RE/LE 126/123 and 122/122 ms, correspondingly), and 2 others had normal latencies (RE/LE in both 114/111 ms). In all four cases with B_{12} hypovitaminosis the dynamics of remyelination were positive (improvement in the range of 11–26 ms), and P1 latency was normal in the second follow-up measurement in all of them. Nine patients (17 %) had vitamin B_1 deficiency (serum concentration of vitamin B_1 under 42 μg dm^{-3}): three of them had abnormal P1 latencies and the response was absent in one patient at the first examination. Only the last patient with an absent evoked response remained without positive dynamics at the second examination, while in three others the P1 latencies recovered to normal figures with a range of improvement of 12–23 ms.

Two patients (4 %) had increased serum levels of thyroid-stimulating hormone (TSH) suggesting low production of thyroid hormone. One of them had only a minor increase of TSH—5.014 mIU dm^{-3} (normal range 0.5–4.9 mIU dm^{-3})—and positive dynamics of P1 latency shortening during the study period from 126/123 ms to normal 111/112 ms for RE/LE. The second patient had 22.69 mIU dm^{-3} TSH, but only one VEP examination performed 2 years after discharge from hospital with normal P1 latencies bilaterally.

Of four patients (7 %) with diabetes mellitus, three patients had hyperglycaemia poorly controlled during the follow-up period: the first patient with serum glucose of 8.7 and 7.6 mmol dm^{-3} measured during the follow-up study became completely blind during the first 24 h of hospitalization with acute methanol poisoning without any dynamics during the 2-year period; the second patient with serum glucose of 5.7 and 8.5 mmol dm^{-3} had P1 latency nonrecordable in the RE without any dynamics and abnormal P1 latency in the LE, which remained abnormal at the second examination, however with certain positive dynamics (shortening from 154 to 130 ms). Finally, in the third patient with serum glucose of 13.8 and 9.2 mmol dm^{-3} measured P1 latencies were normal at the first examination and demonstrated a tendency to further shortening during the study period (114/113–101/100 ms for RE/LE).

Association was present between the dynamics of P1 latency shortening during the study period and serum carbohydrate-deficient transferrin (CDT), the parameter indicating chronic alcohol abuse during the period after acute methanol poisoning. Figure 4 presents the association between the dynamics of P1 latency shortening and concentration of CDT measured during the follow-up clinical examinations. Negative correlation was present between the dynamics of P1 latency shortening and serum concentration of CDT ($r = -0.413$; $p = 0.010$). No correlation of P1 latency dynamics with gamma-glutamyl

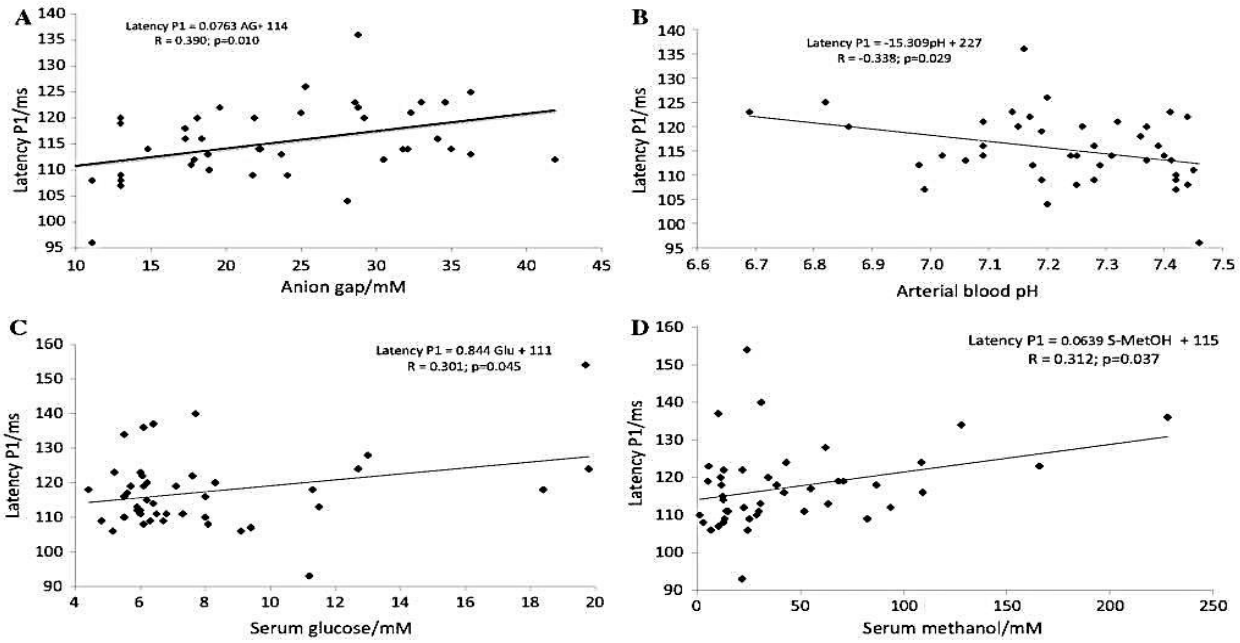

Fig. 2 **a** Anion gap on admission versus latency of P1 peak of evoked responses measured 3–8 months after discharge from hospital. *AG* anion gap. **b** Arterial blood pH on admission versus latency of P1 peak of evoked responses measured 3–8 months after discharge from hospital. **c** Serum glucose concentration on admission versus latency of P1 peak of evoked responses measured 3–8 months after discharge from hospital. *Glu* serum glucose on admission. **d** Serum methanol concentration on admission versus latency of P1 peak of evoked responses measured 3–8 months after discharge from hospital. *S-MetOH* serum methanol concentration on admission

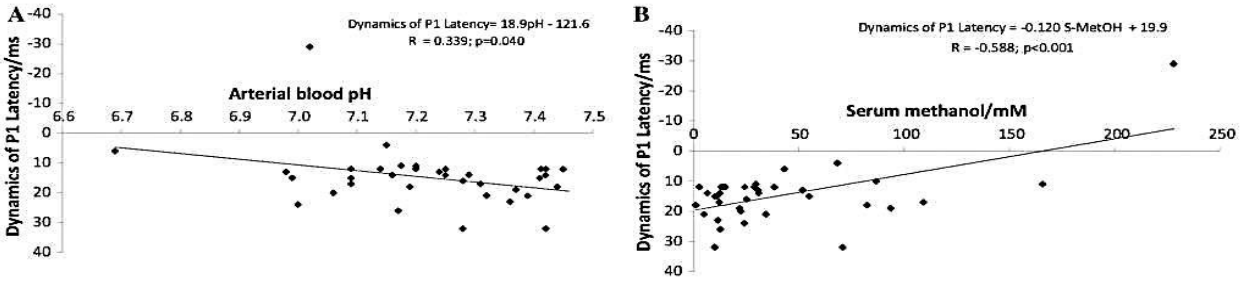

Fig. 3 **a** Arterial blood pH on admission versus dynamics of shortening of latency of P1 peak of evoked response during the study period. **b** Serum methanol concentration on admission versus dynamics of shortening of latency of P1 peak of evoked response during the study period. *S-MetOH* serum methanol on admission

transferase (GGT), TSH, serum glucose, vitamin B_{12}, and vitamin B_1 measured at follow-up examinations were present (all $p > 0.05$).

The main finding of the study was the functional evidence of remyelination of the optic nerve over at least two consecutive years after acute methanol-induced optic neuropathy. The process of remyelination occurred in cases of mild to moderate damage of myelin sheaths of the optic nerve with P1 latency measurable 3–8 months after discharge from hospital. No improvement of conductivity was found in the severe cases with nonrecordable P1 latency at the first VEP examination. Both the severity of initial myelin damage and the dynamics of remyelination were associated with the degree of metabolic acidosis and the severity of poisoning characterized by anion gap, arterial blood pH, serum methanol, and glucose on admission to

Table 3 Biochemical parameters on follow-up examination in 54 patients (means with 95 % confidence interval)

	CDT (1st)/%	CDT (2nd)/%	GGT (1st)/ μkat dm^{-3}	GGT (2nd)/ μkat dm^{-3}	S-glucose (1st)/mmol dm^{-3}	S-glucose (2nd)/mmol dm^{-3}	Vitamin B_{12} (1st)/nmol dm^{-3}	Vitamin B_{12} (2nd)/nmol dm^{-3}	Vitamin B_1/nmol dm^{-3}	TSH (1st)/ mIU dm^{-3}	TSH (2nd)/ mIU dm^{-3}
Total ($n = 54$)	3.0 ± 1.1	2.5 ± 1.1	1.51 ± 0.70	2.1 ± 1.0	5.40 ± 0.47	5.18 ± 0.30	470 ± 170	408 ± 51	59.0 ± 5.1	2.37 ± 0.30	2.74 ± 0.93
Group I ($n = 27$)	3.0 ± 1.6	2.8 ± 2.1	1.7 ± 1.2	2.7 ± 2.0	5.35 ± 0.50	5.29 ± 0.47	570 ± 320	427 ± 87	62.7 ± 8.4	2.37 ± 0.42	2.44 ± 0.49
Group II ($n = 23$)	3.0 ± 1.8	2.4 ± 1.5	1.23 ± 0.55	1.7 ± 1.0	5.46 ± 0.87	5.17 ± 0.47	374 ± 55	384 ± 66	55.2 ± 7.1	2.37 ± 0.45	2.17 ± 0.46
$P_{I/II}$	0.934	0.748	0.462	0.384	0.062	0.728	0.243	0.441	0.177	0.981	0.429

Group I, patients with abnormal P1 latency on first follow-up examination on at least one eye (including the patients with P1 latency not measurable and the absent response); Group II, patients with normal P1 latency measured on first follow-up examination; CDT, carbohydrate deficient transferrin; GGT, gamma-glutamyl-transferase; S-glucose, serum glucose concentration; TSH, thyroid-stimulating hormone; 1st, results of first follow-up examination; 2nd, results of second follow-up examination; *P*, result of Chi square *t* test

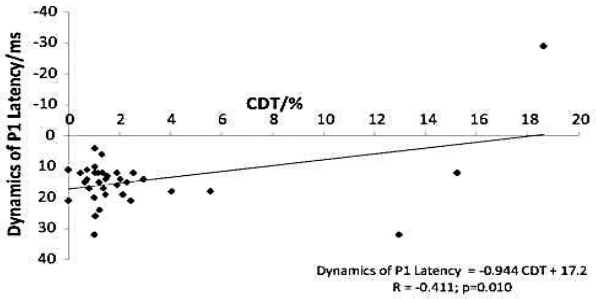

Fig. 4 Serum carbohydrate deficient transferrin concentration on the follow-up examination versus dynamics of shortening of latency of P1 peak of evoked response during the study period. *CDT* serum carbohydrate deficient transferrin (reference limits 0–2 %)

hospital. Chronic alcohol abuse during the period after acute methanol poisoning had a negative effect on the dynamics of remyelination of the optic nerve.

Formic acid, as the major toxic metabolite of methanol in humans, has a myelinoclastic effect causing the swelling and breakdown of myelin sheaths of the optic nerve [9]. During acute poisoning, swelling of damaged myelin sheaths leads to a compression-type injury to the nerve fibers and conduction deficit and block [28, 29]. Conduction block caused by axonal compression normally recovers within a few weeks, and the degree of conductivity delay detected by VEP latency more than 2 months after acute methanol poisoning is most likely a manifestation of the extent of acute demyelination. The direct association between latency delay and the degree of optic nerve demyelination has been confirmed both in animal models and in humans with episodes of acute optic neuritis [30, 31].

In our study, most of the patients (54 %) had abnormal P1 latencies due to demyelination of the optic nerve persisting for more than 2 months after methanol poisoning and discharge from hospital, when the acute symptoms had subsided and the conductivity block caused by edema had disappeared. It is interesting that unilateral damage with abnormal latencies measured in one eye only was not a rare event, occurring in 12 % of cases in our study. Association was present between the biochemical parameters characterizing the degree of metabolic acidosis on admission (anion gap, arterial blood pH) and the extent of myelin damage. It is known that acidosis increases the toxicity of formic acid by enabling greater diffusion of undissociated formic acid into cells [9, 11]. The severity of acidosis depends not only on serum concentration of formic acid but on serum lactate as well [11, 22, 32].

The group of patients with abnormal P1 latency had higher lactacidemia and insignificantly higher serum glucose on admission. Higher serum lactate and glucose could mean deeper and longer hypoxia and more serious damage of both neurons and oligodendrocytes. It is known that serum lactate concentration correlates with clinical outcome in critically ill patients and can be used as a prognostic indicator of mortality and long-term morbidity in critical states [32]. Stress-induced hyperglycemia seen in critically ill patients is also a known prognostic factor of poor outcome [33].

The patients with pathologic P1 latency had significantly higher serum concentrations of methanol on admission than those with normal latencies. In earlier studies, it was shown that serum methanol concentration had no independent prognostic value, but patients with higher serum methanol were commonly more severely poisoned [1, 5, 21]. In our study, serum methanol concentration correlated with the degree of metabolic acidosis, explaining the positive association between this toxicological parameter and P1 latency prolongation.

Several reasons can explain the absence of association between the concentration of serum formic acid on admission and P1 latency. First, the small number of patients with formate measured on admission (27 of 54);

second, most of the patients in the study were so-called "late-presenters" (patients admitted to hospital more than 12 h after ingestion of toxic spirits stopped), so the peak of serum formate concentration and the peak of the toxic effect on optic nerve could have occurred before admission to hospital. Finally, serum formate was measured in different toxicological laboratories in several hospitals throughout the country; therefore, laboratory errors could have taken place or inaccurate registration of the time of blood sampling could have occurred, and samples drawn after the start of dialysis could be considered as the samples drawn on admission.

The possibility of long-term remyelination after acute methanol-induced optic neuropathy can be inferred from studies of acute optic neuritis in patients with multiple sclerosis, but to our best knowledge, studies of its prevalence and dynamics in methanol poisoned patients are absent. In our study, we found a significant decrease in P1 latency over the period of 2 years after discharge from hospital. Quantitatively, the P1 latency reduction was approximately 15–17 ms during the study period, which was comparable with the latency reduction in the 2 years after the acute episode of optic neuritis in the patients with multiple sclerosis studied by Brusa et al. [25, 26]. Although the results of the present study cannot be directly compared with the studies of remyelination in multiple sclerosis, the data suggest that the latency reduction after acute methanol-induced optic neuropathy proceeds for at least 24–28 months, probably at an exponentially decreasing rate with most of the effect during the first year after acute poisoning.

In most of the patients with measurable abnormal P1 latency the rate of remyelination was sufficient to provide a degree of repair for complete restoration of normal conductivity 2 years after methanol poisoning. The dynamics of P1 latency shortening demonstrated an association with the degree of metabolic acidosis and serum methanol concentration on admission, key biochemical and toxicological parameters characterizing the severity of acute poisoning. In the patients with a higher degree of acidosis and serum methanol concentration the dynamics of P1 latency shortening was slower, suggesting a greater extent of demyelinated areas requiring remyelination for the restoration of conductivity. No positive dynamics were present in the most severe cases with P1 latency non-recordable at the first examination. None of these severely poisoned patients showed improvement of conductivity which can be ascribed to neuronal degeneration and loss of most of the axons constituting the optical nerve.

Vitamin B_{12} is known as a co-factor in myelin formation, and its deficiency can lead to focal demyelination of the optic nerve and defective formation of myelin sheaths during the process of remyelination [34]. Deficiency of vitamin B_1 due to chronic alcoholism or other reasons can lead to Wernicke's encephalopathy with possible optic neuropathy and myelination disorders [35, 36]. In our study, no association was found between the serum concentration of vitamin B_{12} measured at both follow-up examinations, and the dynamics of remyelination. No effect of hypovitaminosis B_1 on the dynamics of remyelination was found either. The reasons for the absence of association between the dynamics of remyelination and serum concentrations of both vitamins might be the small number of patients with hypovitaminosis (four and nine cases, correspondingly) and the minor and transient decrease of serum concentrations (the concentrations were close to the lower normal limits; no patients had low serum levels of vitamin B_{12} detected twice during the study).

Hormonal and metabolic disturbances such as hypofunction of the thyroid gland and diabetes mellitus can have an effect on the dynamics of myelin repair. It is known that thyroid hormone is required for normal myelin production and it plays an important part in regulating the lineage and maturation of oligodendrocytes [37]. Diabetes mellitus with insufficiently controlled glycemia may lead to optic nerve neuropathy and even atrophy through vascular and other mechanisms [38]. In our study only two patients had increased serum levels of TSH indicating low thyroid hormone production; therefore it was not possible to study the association between thyroid hormone level and the dynamics of remyelination. In four patients with diabetes three of them had hyperglycemia poorly controlled with episodes of serum glucose registered during the follow-up study higher than 7.0 mmol dm^{-3}. Nevertheless, one of these patients became completely blind during the first day of hospitalization with acute poisoning, and the second one had P1 latency recordable only in one eye, in which some positive dynamics of P1 latency change was present. In the third patient with high glycemia registered at both follow-up examinations, P1 latencies were normal.

Finally, chronic alcohol abuse can negatively affect the process of remyelination due to nutritional deficits and other pathologic mechanisms [39, 40]. Serum CDT is one of the effective markers of heavy chronic alcohol abuse with overall sensitivity of 82 % and specificity of 97 %: alcoholic subjects consuming 50–80 g of alcohol per day for at least a week show increased serum levels of CDT [41]. In our study, the patients with elevated CDT measured at the follow-up examinations demonstrated slower dynamics of P1 latency shortening, which can be suggested as evidence of the negative effect of chronic alcohol abuse on the restoration of conductivity in the optic nerves after acute methanol neuropathy.

Strengths and limitations

The study has some principal limitations, the most important one being the lack of VEP measurement before acute methanol poisoning, during treatment in hospitals, and on discharge from hospital, which prevents the comparison of P1 latencies measured during the follow-up period with the latencies of the "intact" optic nerve before toxic exposure and the latencies at the peak of toxic exposure. The time between discharge from hospital and the follow-up VEP measurements differed in the 54 patients from 3 to 8 months for the first examination and from 24 to 28 months for the second examination, making possible additional inter-individual variability in the results of the latency measurements. The study was not controlled relating to treatment modalities (choice of antidote and mode of dialysis, alkalinization, and folate substitution) which could affect the outcome of methanol poisoning. A substantial number of the survivors of acute poisoning chose not to participate in the follow-up study; therefore, the selection bias could be possible with less severely affected patients prevalently participating in the follow-up examinations.

Despite the limitations and confounders, this is the first prospective study performed after a mass methanol poisoning outbreak comparing the prevalence and dynamics of optic nerve remyelination in surviving patients in association with key toxicological and biochemical parameters measured on admission to hospital and during a 2-year follow-up period, representing the most comprehensive data ever generated in the field. The essential clinical and laboratory data were collected during the Czech mass outbreak in a prospective manner using standardized forms; the follow-up examinations were performed in one medical facility using a uniform investigation protocol.

Methods

Patients

The study was designed as a prospective cross-sectional examiner-masked study. During the Czech mass methanol outbreak, all cases of confirmed methanol poisonings treated in hospitals were documented using a standardized admission protocol, and discharge reports of all hospitalized patients with confirmed diagnosis and results of neurological and ophthalmologic examinations on admission, during hospitalization, and on discharge were collected and analyzed in the Czech Toxicological Information Center.

On admission, the laboratory investigations included serum concentrations of methanol, ethanol, formate, lactate, electrolytes, arterial blood gases, anion and osmolal gaps, glucose, urea, creatinine, bilirubin, liver enzymes, complete blood count, hematocrit, and serum proteins. The diagnosis was established if (1) a history of recent ingestion of illicit spirit was available, and serum methanol concentration was more than 6.2 mmol dm^{-3} (200 mg dm^{-3}), or (2) there was a history/clinical suspicion of methanol poisoning, serum methanol detectable, and at least two of the following were present: pH less than 7.3, serum bicarbonate less 20 mmol dm^{-3} (20 mEq dm^{-3}), and anion gap more than 19 mmol dm^{-3} (19 mEq dm^{-3}). The ophthalmologic examinations during hospitalization and on discharge included fundus examination and standard ophthalmic tests.

The patients were treated in accordance with the American Academy of Clinical Toxicology (AACT) and European Association of Poisons Centres and Clinical Toxicologists (EAPCCT) practice guidelines for the treatment of methanol poisoning [9]. Bicarbonate was given as a buffer to the patients with metabolic acidosis. Ethanol or fomepizole was used as antidote to block alcohol dehydrogenase enzyme. Intermittent hemodialysis (IHD) or continuous veno-venous hemodialysis/hemodiafiltration (CVVHD/HDF) was performed to eliminate formic acid and methanol and correct the metabolic acidosis [42]. Folates were administered in 63 patients: folic acid in 35, and folinic acid in 28 subjects [43]. Corticosteroids were not administered to the patients with visual disturbances.

Laboratory investigations

Methanol was measured by a gas chromatographic method with flame ionization detection and a direct injection with internal standard (Gas Chromatograph Chrom 5, Laboratory Instruments Prague, Czech Republic), limit of detection 60 mg dm^{-3} (1.9 mmol dm^{-3}), and day-to-day coefficient of variation 2.5–5.4 % [44]. Calibrators and controls were made by dilution of methanol p.a. (Penta, Czech Republic). Formate was measured enzymatically on a Hitachi analyzer (Hitachi 912, Hitachi Science Systems Ltd., Japan) using formate dehydrogenase (Roche, France) and nicotinamide adenine dinucleotide (NAD) (Roche, France). Pure sodium formate (Sigma-Aldrich, USA) was used to prepare a standard of 46 mg dm^{-3} (1.0 mmol dm^{-3}) in phosphate buffer and two control sera. Day-to-day coefficient of variation was 5.6 %, and the upper reference limit was 2 mg dm^{-3} (0.44 mmol dm^{-3}). Serum ethanol was analyzed by gas chromatography with flame ionization detection and direct injection with an internal standard (Gas Chromatograph Chrom 5, Laboratory Instruments Prague, Czech Republic). The limit of detection was 40 mg dm^{-3} (0.87 mmol dm^{-3}), and the day-to-day coefficient of variation was 3.8–7.1 % [45].

Ethanol standards were purchased (Erba Lachema, Czech Republic).

The anion gap in serum was determined from the equation:

$$AG = (Na^+ + K^+) - (Cl^- + HCO_3^-)$$

where AG—anion gap, Na^+—serum concentration of sodium, K^+—serum concentration of potassium, Cl^-—serum concentration of chlorine, HCO_3^-—serum concentration of bicarbonate. The reference range is $13 + 8$ mmol dm^{-3} (mean + 2SD) [46].

Clinical investigation protocol

The clinical examination protocol 3–8 months (first examination) and 24–28 months (second examination) after discharge from hospitals included complete ocular examination and standard ophthalmic tests (visual acuity, color vision, visual fields, contrast sensitivity, fundoscopy), full-field VEPs, biochemical tests [electrolytes, glucose, glycohemoglobin, albumin, pre-albumin, urea, creatinine, bilirubin, liver enzymes, cholesterol, lipids, thyroid-stimulating hormone (TSH), vitamin B_{12}, vitamin B_1, carbohydrate deficient transferrin], qualitative ethyl glucuronide screening in urine, and standardized questionnaire forms. The examiners were masked to the serum methanol and formic acid concentrations on admission, severity of poisoning, clinical course, treatment measures and outcomes in methanol-poisoned patients on discharge from hospitals, as well as to each other's results.

VEP examination was performed on two-channel device TruTrace 4 Alien Technik CZ. Monocular checkerboard pattern-reversal stimulation was used, with frequency of 1.5 c s^{-1}, angular size of the monitor 6° × 5° from the fixation point, angular size of checkerboard squares 40′. Luminance of the white and black squares was 84 and 57 cd m^{-2}, respectively. Bandwidth of the amplifier was 1 Hz–1 kHz, the evoked response was registered from the Oz–Fz derivation. At each eye, the examination was performed twice in order to check reproducibility of the evoked complex. We evaluated latencies of waves N1, P1, and N2. The measured values in patients were compared with our laboratory reference values determined as the central 95 % interquantile interval of data measured on a group of 30 healthy individuals. Four criteria of latency abnormality were chosen: (1) wave N1 latency >88 ms, (2) wave P1 latency >117 ms, (3) wave N2 latency >160 ms, and (4) absent response. The result was categorized as abnormal if at least one of the above-mentioned criteria was fulfilled. As long as the latency of P1 peak of evoked response (the first major positive component of the wave complex) is characterized by the best relation between the sensitivity and inter-individual variability, this parameter was used for the further analysis of association between the dynamics of remyelination and the biochemical determinants [47].

Statistical analyses

The admission laboratory data in the different groups were compared on a group by group basis using two-sample t test assuming unequal variances (equal means), two-sample F test for variances, bias test, and two-sample Kolmogorov–Smirnov test. Data are expressed as arithmetic means with confidence intervals. For comparison of the obtained results, common statistical tests have been used [t test: two-sample assuming equal variances, t test: two-sample assuming unequal variances (equal means), two-sample F-test for variances, bias test, and ANOVA]. Chi square tests were used to examine the differences between the groups with normal and abnormal P1 latencies. Pearson's correlation and linear regression analysis were used to examine the relationships between various parameters. All statistical calculations were carried out with a level of significance $\alpha = 0.05$. Statistical documentation was performed in Excel (Microsoft, USA), and the formal calculations were produced in QC Expert software 3.1 (Trilobyte, Pardubice, Czech Republic) and in IBM SPSS ver. 17.0 and Statistica SF ver. 10.0 (both licensed to 1st Faculty of Medicine of Charles University in Prague).

Ethics

The study was approved by the General University Hospital Ethics Committee in Prague, Czech Republic. Informed consent was obtained from the participants of the study. The study adhered to the tenets of the Declaration of Helsinki.

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Clinical and genetic determinants of chronic visual pathway changes after methanol - induced optic neuropathy: four-year follow-up study.

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Clinical and genetic determinants of chronic visual pathway changes after methanol - induced optic neuropathy: four-year follow-up study

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ABSTRACT

Context: Methanol poisoning induces acute optic neuropathy with possible long-term visual damage.

Objective: To study the dynamics and key determinants of visual pathway functional changes during 4 years after acute methanol poisoning.

Methods: A total of 42 patients with confirmed methanol poisoning (mean age 45.7 ± 4.4 years) were examined 4.9 ± 0.6, 25.0 ± 0.6, and 49.9 ± 0.5 months after discharge. The following tests were performed: visual evoked potential (VEP), retinal nerve fiber layer (RNFL) measurement, brain magnetic resonance imaging (MRI), complete ocular examination, biochemical tests, and apolipoprotein E (ApoE) genotyping.

Results: Abnormal VEP P1 latency was registered in 18/42 right eyes (OD) and 21/42 left eyes (OS), abnormal N1P1 amplitude in 10/42 OD and OS. Mean P1 latency shortening during the follow-up was 15.0 ± 2.0 ms for 36/42 (86%) OD and 14.9 ± 2.4 ms for 35/42 (83%) OS, with maximum shortening up to 35.0 ms. No significant change of mean N1P1 amplitude was registered during follow-up.

A further decrease in N1P1 amplitude ≥1.0 mcV in at least one eye was observed in 17 of 36 patients (47%) with measurable amplitude (mean decrease −1.11 ± 0.83 (OD)/−2.37 ± 0.66 (OS) mcV versus −0.06 ± 0.56 (OD)/−0.83 ± 0.64 (OS) mcV in the study population; both $p < .001$).

ApoE4 allele carriers had lower global and temporal RNFL thickness and longer initial P1 latency compared to the non-carriers (all $p < .05$). The odds ratio for abnormal visual function was 8.92 (3.00–36.50; 95%CI) for ApoE4 allele carriers ($p < .001$). The presence of ApoE4 allele was further associated with brain necrotic lesions ($r = 0.384$; $p = .013$) and brain hemorrhages ($r = 0.395$; $p = .011$).

Conclusions: Improvement of optic nerve conductivity occurred in more than 80% of patients, but evoked potential amplitude tended to decrease during the 4 years of observation. ApoE4 allele carriers demonstrated lower RNFL thickness, longer P1 latency, and more frequent methanol-induced brain damage compared to non-carriers.

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KEYWORDS Toxic optic neuropathy; methanol poisoning; chronic axonal neurodegeneration; remyelination; visual evoked potential; long-term visual sequelae; apolipoprotein E

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Introduction

Methanol is one of the most widely applied toxic alcohols in industry, agriculture, and households. It is used as an organic solvent, biofuel, antifreeze fluid component, for car chemistry, and in the production of other chemical substances and compounds. Acute or subacute exposure to methanol leads to systemic intoxication and development of toxic optic neuropathy. Methanol poisoning outbreaks due to consumption of illicit alcoholic drinks present a challenge for healthcare systems throughout the world due to a high lethality rate and serious visual and central nervous system (CNS) damage in survivors [1–5]. Treatment involves inhibition of methanol transformation to the highly toxic formic acid by alcohol dehydrogenase (ADH), with ethyl alcohol or 4-methylpyrazole [6–9]. Formic acid accumulation results in mitochondrial cytochrome *c* oxidase inhibition, impairment of oxygen utilization, membrane lipid peroxidation, depletion of ATP in cells, and severe metabolic acidosis [10–13].

The ocular retina is one of the tissues with the highest oxygen consumption, and the axons of retinal ganglion cells, which form the optic nerve, are selectively vulnerable to histotoxic hypoxia caused by formic acid because of their high energy dependence [14–16]. The symptoms of methanol-induced optic neuropathy manifest after a latency period of 6 to 48 h, depending upon the amount of methanol ingested, possible ethanol co-ingestion, and body mass. Symptoms range from blurred or "snowfield" vision, reduced visual acuity (VA), photophobia, peripheral constriction of visual fields, central/centrocecal scotomata, loss of color vision, and complete blindness. In many cases, restoration of visual functions with the resolution of pathologic changes to the fundus and improvement of VA occurs 1–2 months after methanol exposure. However, long-term visual impairment may be present in 25–40% of patients [17–19]. It is difficult to predict the character and degree of long-term visual damage in patients with acute or subacute methanol poisoning and new or initially unrecognized visual disturbances may manifest several years after poisoning [20].

Acute demyelination of the optic nerve due to the toxic effects of formic acid may result in axonal degeneration due to the lack of trophic support from myelin and disruption of normal axon–myelin interactions. Optic neuritis studies demonstrate that spontaneous remyelination takes place after the acute episode of demyelination with a visual evoked potential (VEP) latency shortening of 6–7 ms during the first 6 months and a further reduction of 4 ms between 6 months and 2 years after the first attack [21,22]. VEP measurements provide important data on the dynamics of chronic optic nerve conductivity changes associated with myelin repair and restoration of visual pathway integrity. The prolongation of the wave P1 latency of full-field VEP reflects the degree of demyelination of the optic nerve fibers, and a decrease in N1P1 amplitude reflects the number of impaired axons after acute damage [23].

Knowing the character and severity of visual pathway damage, recognizing long-term visual sequelae, and understanding the key determinants of chronic visual impairment during the years following discharge from hospital is important to evaluate the effectiveness of therapeutic interventions, prognosis, and timely indication of special devices to enhance the quality of life of methanol poisoning survivors. Nevertheless, there are no prospective longitudinal cohort studies on the prevalence, character, and dynamics of chronic functional changes of the visual pathway after methanol-induced optic neuropathy with the series of complete ophthalmological examinations, VEP, optical coherence tomog-

raphy (OCT) of peripapillary retinal nerve fiber layer (RNFL) thickness, and magnetic resonance imaging (MRI) of the brain performed several times during the years following acute poisoning.

We present the data based on a methanol mass poisoning outbreak with more than 130 cases of poisoning and more than 50 deaths [24]. In 2013, we performed a cross-sectional study of the prevalence and character of visual damage in the survivors of poisoning after discharge from hospital [18]. During the 4 years after the methanol poisoning "epidemic", we carried out three consecutive clinical examinations of the survivors according to the standardized clinical protocol in one medical facility to determine the dynamics of chronic changes of the visual pathway and its association with key clinical, genetic, and laboratory parameters measured during hospitalization and the follow-up observation period.

Materials and methods

Study design and setting

The prospective longitudinal cohort study included patients with confirmed methanol poisoning treated in 30 hospitals in the country during a mass methanol poisoning outbreak [18,24]. The initial clinical, toxicological, and biochemical data were obtained from treatment providers by applying a standardized data collection form and sent to the Toxicological Information Center (TIC) on the day following hospital admission. Information on pre-hospital and hospital therapeutic interventions and the outcomes was obtained from hospital discharge reports. The follow-up clinical examinations were performed three times in the same hospital approximately 6, 24, and 48 months after discharge from hospital. The study was approved by the General University Hospital Ethics Committee in Prague, Czech Republic.

Selection of participants and treatment

Mandatory reporting to the Ministry of Health and TIC on all cases of hospital admission with acute methanol poisoning and nationwide daily monitoring of the situation in all hospitals was established. All patients hospitalized with confirmed methanol poisoning were eligible for this study.

For controls, healthy subjects of the same age, ethnicity, and history of chronic alcohol abuse were recruited. Exclusion criteria for the controls were intraocular pressure ≥22 mmHg or glaucoma in either eye; evidence of a reproducible visual field (VF) defect (pattern standard deviation significant at the <5% level or abnormal glaucoma hemifield test result) in either eye as measured with Medmont automated perimeter M700 (Medmont International Pty Ltd, Vermont, Australia); unreliable VFs (false-positive or false-negative rate >15% or fixation losses >20%); any pattern of loss consistent with ocular disease; intraocular surgery in the study eye (except cataract or refractive surgery if performed more than one year before testing); best-corrected VA worse than 20/32 on the Early Treatment Diabetic Retinopathy Study scale; evidence of diabetic retinopathy, diabetic macular edema, or other vitreoretinal disease in either eye; evidence of optic nerve or RNFL abnormality in either eye; and use of a photosensitizing agent within 14 days.

Patients with methanol poisoning were treated in accordance with the American Academy of Clinical Toxicology (AACT) and European Association of Poisons Centres and Clinical Toxicologists (EAPCCT) practice guidelines for the treatment of methanol poisoning [25]. Bicarbonate was administered as a buffer to patients with severe metabolic acidosis on admission. Ethyl alcohol or 4-methylpyrazole was used as an antidote to inhibit methanol oxidation by ADH [26,27]. Intermittent or continuous modalities of renal replacement therapy were applied in severely poisoned patients to eliminate formate and methanol and to correct acidemia [28,29]. Folic or folinic acid was administered to substitute the internal folate pool [30].

Clinical examinations and laboratory analyses during hospital treatment

Detailed histories of poisoning and of ocular and systemic toxicity were received from the hospitalized patients or from relatives of critically ill patients upon admission to hospital. Diagnosis was established when (i) a history of recent ingestion of illicit spirits was available and serum methanol was higher than 200 mg/L, or (ii) there was a history or clinical suspicion of methanol poisoning, and serum methanol was above the lower limit of detection with at least two of the following: pH <7.3, serum bicarbonate <20 mmol/L, or anion gap (AG) ≥ 20 mmol/L [25].

The standardized protocol of clinical examinations included ocular examination with standard ophthalmologic tests (VA, color vision, VFs, contrast sensitivity, and fundus examination), cerebral computed tomography (CT) or MRI of the brain, and standard neurological examination. Patients were considered to have visual sequelae of acute methanol

poisoning if the symptoms of toxic optic neuropathy were documented on admission and/or during hospitalization, with pathologic findings on VA, perimeter, color vision, contrast sensitivity, and persisting lesions on fundoscopy on discharge from hospital. Further, patients were considered to have CNS sequelae of poisoning if symmetrical necrosis and hemorrhages of basal ganglia and subcortical white matter compatible with the diagnosis of acute methanol poisoning were present on CT or MRI of the brain.

Apolipoprotein E (ApoE) genotyping

DNA was isolated from frozen ethylenediaminetetraacetic acid (EDTA) blood using a modified method according to Miller et al. [31]. ApoE genotyping was performed using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis. The oligonucleotides 5′-ACA GAA TTC GCC CCG GCC TGG TAC AC-3′ and 5′-TAA GCT TGG CAC GGC TGC CAA GGA-3′ were used to amplify a 244 base pair (bp) fragment of the *ApoE* gene. CfoI restriction enzyme (Fermentas) was used to cut the PCR product. Restriction fragments (separated on a 15% polyacrylamide gel) of 91 bp and 83 bp are typical for the *ApoE2* allele, fragments of 91 bp and 48 bp for the *ApoE3* allele, and 72 bp and 48 bp fragments for the *ApoE4* allele. Known ApoE genotype frequencies of a large group of an adult elderly population (HAPIEE study, $n = 6230$) were used for comparison [32].

Follow-up clinical examination protocol

The follow-up clinical examination protocol was previously described in detail [33–35]. The study protocol included complete ocular examination and standard ophthalmic tests, including VA measurement, slit-lamp examination, intraocular pressure measurement, fundus examination, color vision, VFs, OCT with RNFL, VEP, MRI of the brain, neurological and neuropsychological examinations, biochemical tests (glucose, glycohemoglobin, hepatic tests, cholesterol, lipids, thyroid-stimulating hormone (TSH), vitamins B_{12} and B_1, carbohydrate-deficient transferrin, ethyl glucuronide (in urine), and standardized questionnaire forms.

The VEP examination was performed on a two-channel TruTrace 4 Alien Technik CZ device. Monocular full-field checkerboard pattern-reversal stimulation was used, with the reversal frequency of 1.5 c/s, angular size of the monitor 6°×5° from the fixation point, and angular size of checkerboard squares was 40′. Luminance of the white and black squares was 84 cd/m^2 and 57 cd/m^2, respectively, corresponding to the contrast between black and white squares of about 20%. Bandwidth of the amplifier was 1 Hz–1 kHz, and the evoked response was registered from the Oz-Fz derivation. For each eye, examination was performed twice in order to check reproducibility of the evoked complex. We evaluated the latency of the P1 wave and the N1P1 amplitude. Four criteria of abnormality were chosen: (1) missing evoked response, (2) P1 wave latency >117 ms, (3) interocular difference of P1 wave latencies >6 ms, and (4) amplitude of evoked complex <3 μV. The result was categorized as abnormal if at least one of the above-mentioned criteria was fulfilled.

The medical examiners which performed the follow-up clinical examinations were masked to the results of toxicological and biochemical measurements, severity of poisoning, clinical course, treatment measures, and clinical outcomes upon discharge from hospital.

Calculations and data analysis

Basic descriptive statistics (mean, median, confidence interval (CI), SD, skewness, and kurtosis) were computed for all variables, which were subsequently tested for normality using the Kolmogorov–Smirnov test. The Chi-squared (χ^2) test was used to compare frequency counts of demographic and clinical categorical variables. The bivariate relationship was assessed using Pearson's correlation coefficient. A linear mixed effects model was applied to study the longitudinal relationship between demographic, clinical, and laboratory parameters, and the results of VEP P1 latency measurements during the study period. The dependent variable in this model was P1 latency measured on both eyes (OD, OS – two measurements) over time (three measurements during four years), resulting in six measurements per individual. The results of repeated measurements were nested for each individual and an autoregressive covariance structure (AR-1) was used to model the relationships between observed variables. The independent variables included in the model were: age, sex, time, severity of metabolic acidosis (arterial blood pH), acute serum concentrations of methanol and ethanol on admission, and follow-up serum glucose, vitamins B_1 and B_{12}, and TSH measured during the

study period. Due to the low number of ApoE2/E2 and ApoE4/E4 homozygotes, these individuals were pooled with adequate heterozygotes. Thus, three groups (ApoE2/E2 + ApoE3/E2 versus ApoE3/E3 versus ApoE4/E3 + ApoE4/E4) were compared.

Statistical significance was set at $p < .05$. Statistical analysis was performed in Excel (Microsoft, Redmond, WA), and formal calculations were produced in QC Expert software 3.1 (Trilobyte, Pardubice, Czech Republic) and IBM SPSS version 24.0 (Chicago, IL).

Results

Demographic and clinical data of the study population and the control group

Altogether, 108 patients were admitted to the hospital during a methanol mass poisoning outbreak. Twenty-four patients died during hospitalization and 84 patients survived and were approached and invited to join the group of volunteers in a prospective study of long-term health sequelae at discharge from the hospital. Fifty-five subjects with a mean age at discharge of 46.7 ± 3.6 years (46 men and 9 women) expressed their consent and were examined at least once during the observation period. In 49 patients, ApoE genotyping was performed. The follow-up clinical examinations were performed 4.9 ± 0.6 months, 25.0 ± 0.6 months, and 49.9 ± 0.5 months after discharge from hospital (means with standard deviation (SD)).

Of the 55 patients included in the study, eight subjects died before the third round of clinical examination, and in 5 patients less than three examinations were carried out because of a delay in joining the study program. These 13 patients were excluded from analysis of chronic visual pathway functional changes. Forty-two subjects, 34 men and eight women, underwent three consecutive clinical examinations during 4 years of observation. The control group of 41 individuals was examined according to the same clinical investigation protocol. Thus, 84 eyes from survivors of acute methanol poisoning and 82 eyes from controls were analyzed.

Demographic and clinical laboratory parameters of the studied population and controls are shown in Table 1. Patients exposed to methanol had higher follow-up glycemia because of the five subjects in this group with diabetes mellitus type 2. Blood cholesterol, but not triglycerides, was higher in the study population. The serum level of the vitamin B_1 was relatively higher in the study population, but no patients with hypovitaminosis of B_1 or B_{12} were present in either group.

Table 1. Demographic and laboratory data of the study population and the control group (means with SD).Table Layout

Parameter	Survivors of methanol poisoning ($n = 42$)	Controls ($n = 41$)	p
Age, years	45.7 ± 4.4	44.0 ± 4.2	.73
Chronic alcoholism	23 (55%)	24 (59%)	.43
S-MetOH[a], mg/L	1430 ± 470	–	–
Arterial pH[a]	7.21 ± 0.10	–	–
Base deficit[a], mmol/L	16.5 ± 3.5	–	–
S-Formate[a], mg/L	600 ± 150	–	–
Glucose, mmol/L	5.21 ± 0.29	4.60 ± 0.31	**.004**
ClycHbA1, mmol/mol	33.9 ± 2.1	36.0 ± 1.5	.10
Creatinine, mcmol/L	79.5 ± 4.5	76 ± 9.4	.51
Cholesterol, mmol/L	5.40 ± 0.40	4.61 ± 0.41	**.008**
Lipids, mmol/L	1.52 ± 0.31	1.6 ± 1.0	.86
GGT, ukat/L	1.91 ± 0.80	0.60 ± 0.29	**.006**
TSH, mIU/L	2.30 ± 0.31	2.11 ± 0.60	.55
Vitamin B_1, 1st exam, mcg/L	60.8 ± 5.5	45.7 ± 4.2	**.001**
[a]measured at admission to hospital with acute poisoning			
$p < .05$ was considered significant (bold figures).			

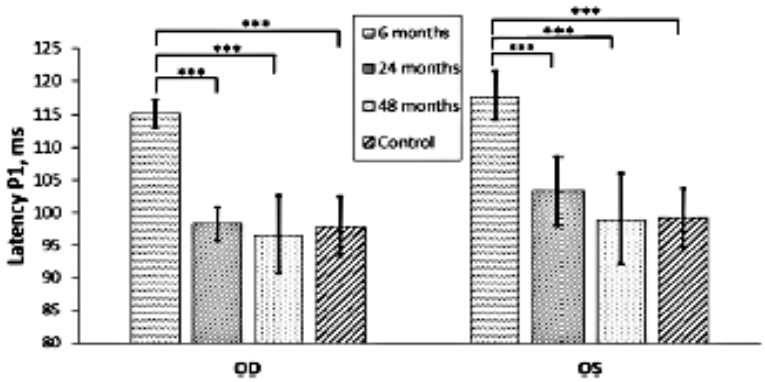
Parameter	Survivors of methanol poisoning ($n = 42$)	Controls ($n = 41$)	p
Vitamin B_1, 2nd exam, mcg/L	53.9 ± 4.5	45.7 ± 4.2	**.009**
Vitamin B_{12}, 1st exam, mcg/L	460 ± 190	420 ± 110	.68
Vitamin B_{12}, 2nd exam, mcg/L	394 ± 51	420 ± 110	.69
Vitamin B_{12}, 3rd exam, mcg/L	423 ± 59	420 ± 110	.93
[a]measured at admission to hospital with acute poisoning			
$p < .05$ was considered significant (bold figures).			

The median ingested volume of toxic alcoholic drinks was 0.3 L (range 0.1–1.5 L) in the study population with approximately 50% methanol and 50% ethyl alcohol in various strong beverages with total alcohol content of approximately 40% by volume. Pre-hospital ethyl alcohol was administered as a "first aid antidote" by the ambulance medical or paramedic staff in 15 (36%) patients. Twenty-six per cent of the patients were admitted to hospital within 12 hours of methanol ingestion, 60% were admitted within 48 hours, and 10% were admitted more than 48 hours after ingestion. Twenty-two patients had severe acidemia on admission, 10 patients were hospitalized in a comatose state (Glasgow coma scale (GCS) of eight or lower), and 20 patients reported at admission subjective signs of visual impairment ranging from blurred and "snowfield" vision to complete blindness (three cases).

Dynamics of the P1 latency of VEPs during the observation period

The results of the measurements of the wave P1 latency of VEP during 4 years of observation are shown in Figure 1. On first examination approximately 6 months after discharge, abnormal P1 latency was measured in 18/42 (43%) right eyes (oculus dexter, OD) and 21/42 (50%) left eyes (oculus sinister, OS), including five OD and four OS without elicitable evoked potential. Significant mean P1 latency shortening, with the most remarkable changes between the first and second examinations, or 2 years after methanol exposure, was observed in the study population with a return of the initially prolonged mean P1 latency to values similar to those measured in the control group (Figure 1).

Figure 1. P1 latency of visual evoked potentials in the study population ($n = 42$) versus controls ($n = 41$). OD: oculus dexter; OS: oculus sinister; ms: : milliseconds; "6 months", "24 months", "48 months": clinical examinations performed after discharge from hospital; ***$p < .001$; **$p < .01$; *$p < .05$.



Mean P1 latency shortening in the study population was 15.0 ± 2.0 ms for 36/42 (86%) OD and 14.9 ± 2.4 ms for 35/42 (83%) OS, with maximum individual changes up to 29.0–35.0 ms. However, in five OD and four OS, visual potential could not be evoked, and in one OD and three OS, the evoked potential became non-elicitable at the second or the third examination. Worsening dynamics and non-elicitable VEP were registered in the most severely poisoned patients with pronounced acidemia on admission to hospital.

Arterial blood pH measured on admission (severity of acidemia) was significantly associated with P1 latency measured at the first and second examinations, 6 and 24 months after discharge ($r_1 = -0.349$; $p = .04$, and $r_2 = -0.379$; $p = .$

02), but not at the third one, four years after discharge, when optic nerve remyelination was completed. There was a similar association between serum methanol concentration and P1 latency: $r_1 = 0.324$; $p = .04$, $r_2 = 0.526$; $p < .001$ and $r_3 = 0.141$; $p = .41$. Finally, there was a positive association between P1 latency measured at the first and second examinations and patient age ($r_1 = 0.509$; $p = .002$, $r_2 = 0.494$; $p = .003$). There were no associations for either P1 latency or the rate of latency shortening with sex or the follow-up laboratory parameters (serum glucose, cholesterol, lipids, vitamins B_1, B_{12}, TSH, and others). However, the rate of P1 latency shortening was slower in patients with high gamma glutamyl transferase (GGT) liver enzyme ($r = 0.359$; $p = .03$).

Regarding treatment modalities, the type of antidote administered in the hospital (ethanol or 4-methylpyrazol) was not significant for P1 latency, but patients treated with continuous modalities of hemodialysis had a longer P1 latency measured at the first and second examinations ($r_1 = 0.343$; $p = .03$ and $r_2 = 0.34$; $p = .04$). The effect of folate substitution on the dynamics of P1 latency was not significant.

For mixed model regression analysis of the dynamics of P1 latency during the observation period, the following parameters were included as predictors: age, sex, time of the follow-up examination, time between methanol exposure and hospital admission, side (reflecting interocular OD/OS P1 latency difference), acute laboratory parameters on admission (arterial blood pH, serum methanol, ethanol, and glucose), vitamins B_1 and B_{12}, and TSH measured during the follow-up period. The results of regression analysis of the variables significant to P1 latency dynamics are presented in Table 2. The observed data of P1 latency dynamics (summarized as means and SD) for arterial blood pH groupings (three groups: severe acidosis, pH ≤7.0; moderate acidosis, pH >7.0 ≤ 7.2; minor acidosis, pH >7.2 ≤ 7.4) over four years of observation are presented in Figure 2.

Figure 2. Dynamics of P1 latency changes during the observation period depending on arterial blood pH at admission. OD: oculus dexter; OS: oculus sinister; Latency: : mean P1 latency, ms; pH: arterial blood pH measured on admission to hospital.

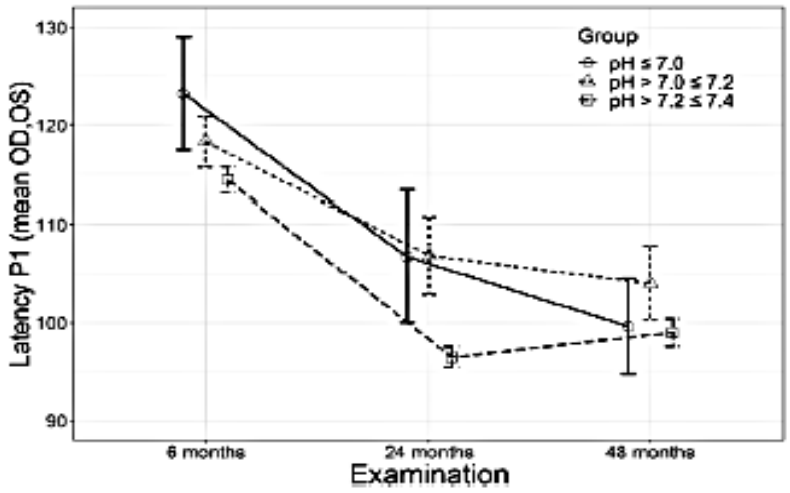


Table 2. Mixed effect model predicting changes of P1 latency during four years of observation.Table Layout

Parameter	Estimate	Standard error	df	t	Significance	95% Confidence interval	
						Lower	Upper
Intercept	262.87	68.89	22.13	3.82	0.00	120.06	405.69
Time of examination: time of the follow-up clinical examination (t_1: 6 months, t_2: 24 months, t_3: 48 months after discharge from hospital); Time^2: time from methanol exposure to hospital admission (a square reflects the non-linear trend); Side OD/OS: parameter reflecting interocular difference in the mean P1 latency (OD = 1, OS = −1); pH: arterial blood pH on admission; MetOH: serum methanol concentration; EtOH: serum ethanol concentration; Glucose: serum glucose concentration; B_1: serum vitamin B_1 concentration; B_{12}: serum vitamin B_{12} concentration; TSH: serum thyroid-stimulating hormone concentration; 1st, 2nd, 3rd: follow-up clinical examinations.							

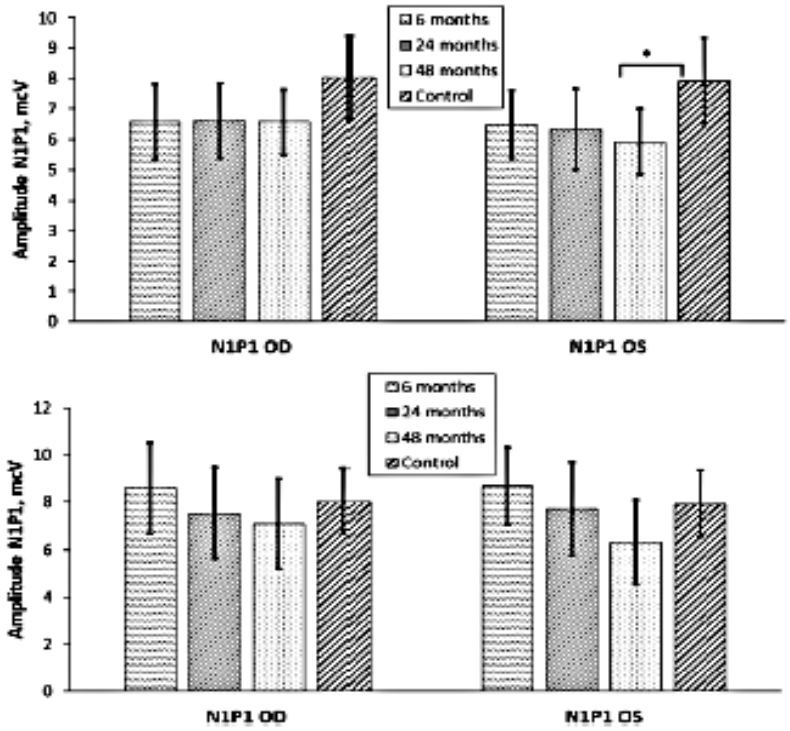
Parameter	Estimate	Standard error	df	*t*	Significance	95% Confidence interval	
						Lower	Upper
Time of examination, t	−12.43	0.53	72.95	−23.27	0.00	−13.49	−11.36
$Time^2$	2.14	0.12	58.51	17.70	0.00	1.90	2.38
Side OD/OS	−0.99	0.38	43.69	−2.66	0.01	−1.75	−0.24
Sex	3.21	2.04	20.98	1.57	0.13	−1.04	7.46
Age, years	0.41	0.12	21.96	3.31	0.00	0.15	0.66
pH	−24.54	9.35	21.99	−2.63	0.02	−43.92	−5.16
MetOH, mg/L	0.00	0.00	21.51	−1.31	0.21	−0.01	0.00
EtOH, mg/L	0.00	0.00	22.61	1.22	0.24	0.00	0.01
Glucose, mmol/L	0.00	0.12	21.49	0.02	0.98	−0.24	0.24
B_1, mcg/L_1st	0.26	0.12	22.27	2.12	0.05	0.01	0.51
B_1, mcg/L_2nd	−1.11	0.69	78.53	−1.62	0.11	−2.48	0.26
TSH, mIU/L	0.05	0.15	66.64	0.35	0.73	−0.24	0.34
B_{12}, mcg/L_1st	0.00	0.00	22.01	−0.09	0.93	−0.01	0.00
B_{12}, mcg/L _2nd	0.01	0.02	21.33	0.50	0.62	−0.03	0.05
B_{12}, mcg/L _3rd	0.00	0.02	21.19	−0.08	0.94	−0.04	0.04

Time of examination: time of the follow-up clinical examination (t_1: 6 months, t_2: 24 months, t_3: 48 months after discharge from hospital); $Time^2$: time from methanol exposure to hospital admission (a square reflects the non-linear trend); Side OD/OS: parameter reflecting interocular difference in the mean P1 latency (OD = 1, OS = −1); pH: arterial blood pH on admission; MetOH: serum methanol concentration; EtOH: serum ethanol concentration; Glucose: serum glucose concentration; B_1: serum vitamin B_1 concentration; B_{12}: serum vitamin B_{12} concentration; TSH: serum thyroid-stimulating hormone concentration; 1st, 2nd, 3rd: follow-up clinical examinations.

Dynamics of N1P1 amplitude of VEPs during the observation period

The results of three consecutive measurements of N1P1 amplitude of VEPs in the study population are presented in Figure 3(A). During the first examination, 6 months after discharge, abnormal N1P1 amplitude was measured in 10/42 (24%) OD and 10/42 (24%) OS, including five OD and four OS without elicitable evoked potential. At the third examination, four years after discharge, abnormal N1P1 amplitude was measured in 10/42 (24%) OD and in 14/42 (33%) OS, including six OD and seven OS without elicitable evoked potential. In one OD and three OS with abnormal N1P1 amplitude measured at the first examination, the evoked potential became non-elicitable during the second or the third examination.

Figure 3. (A) N1P1 amplitude of VEPs in the study population ($n = 42$) versus control group ($n = 41$). (B) N1P1 amplitude of VEPs in the patients with the amplitude of at least 1.0 mcV in at least one eye during the observation period ($n = 17$) versus control group ($n = 41$). OD: oculus dexter; OS: oculus sinister; ms: milliseconds; "6 months", "24 months", "48 months": clinical examinations performed after discharge from hospital; ***$p < .001$; **$p < .01$; *$p < .05$.



Mean N1P1 amplitude in the study population was lower than in the controls; however, the difference did not reach statistical significance (first examination: 6.6 ± 1.2 versus 8.0 ± 1.4 (OD); 6.5 ± 1.1 versus 7.9 ± 1.4 mcV (OS); second examination: 6.6 ± 1.2 versus 8.0 ± 1.4 (OD); 6.3 ± 1.3 versus 7.9 ± 1.4 mcV (OS); third examination: 6.6 ± 1.1 versus 8.0 ± 1.4 (OD); 5.9 ± 1.1 versus 7.9 ± 1.4 mcV (OS); all $p > .05$). No significant changes in mean N1P1 amplitude were registered during the observation period in the study population (Figure 3(A)). The mean decrease of amplitude was −0.06 ± 0.56 mcV for OD and −0.83 ± 0.64 mcV for OS.

In 17 patients from the study population, a decrease in amplitude of at least 1.0 mcV in at least one eye was observed during the observation period. In these 17 patients, the results of three consecutive measurements of N1P1 amplitude of VEPs are presented in Figure 3(B). The mean amplitude decrease in this group ($n = 17$) was −1.11 ± 0.83 mcV for OD and −2.37 ± 0.66 mcV for OS, both of which were significantly higher than for the whole study population ($p < .001$). In eight of 17 patients, an amplitude decrease of at least 1.0 mcV was observed in both eyes, and in nine of them, the decrease occurred only in one eye, predominantly the left one. The highest rate of decrease was 4.6 mcV over 4 years for the right eye and 6.6 mcV for the left eye. None of the patients with a significant amplitude decrease exhibited abnormal N1P1 amplitude at the first examination, and only 3/17 had abnormal amplitude measured at the third examination. The remaining patients (14 of 17) had N1P1 amplitude values within the normal range during the entire observation period.

The negative association between N1P1 amplitude and P1 latency of VEP was strongest at the first examination, six months after discharge, less strong, but still significant, at the second examination, two years after discharge, and was not significant during the third examination, four years after discharge (for OD/OS: $r_1 = -0.398/-0.628$; $p = .02/.000$; $r_2 = -0.354/-0.510$; $p = .037/.001$; and $r_3 = 0.123/-0.036$; $p = .47/.84$). Patient age negatively correlated with N1P1 amplitude, and this correlation was nearly unchanged during 4 years of observation (for OD/OS: $r_1 = -0.469/-0.474$; $p = .004/.003$; $r_2 = -0.436/-0.448$; $p = .009/.006$; $r_3 = -0.454/-0.422$; $p = .005/.01$).

Patients with lower N1P1 amplitude were more severely poisoned and exhibited a lower GCS ($r = 0.418$; $p = .009$) and higher serum methanol concentration at admission to hospital ($r = -0.414$; $p = .01$). The effect of the type of antidote administered in the hospital on N1P1 amplitude was not significant, but patients treated with continuous modalities of hemodialysis had lower amplitude measured during the first and second examinations ($r_1 = -0.495$; $p = .002$, $r_2 = -0.382$;

$p = .02$) compared to intermittent hemodialysis. The effect of folate substitution during treatment on N1P1 amplitude was not significant.

Chronic visual pathway changes and the effect of ApoE gene polymorphism

For the analysis of possible effects of ApoE gene polymorphism on the chronic visual pathway functional changes, 49 patients (98 eyes) with confirmed acute methanol poisoning, including 41 patients (82 eyes) with three follow-up examinations, from the study population were genotyped. Frequencies of individual ApoE genotypes in the study population did not differ significantly from the frequencies in the general Czech population (Table 3).

Table 3. Apolipoprotein E genotype and allele distribution between the study population and general czech population[a].Table Layout

	Patients		Controls		
Genotype	*n*	%	*n*	%	*p*
ApoE2/E2	1	2.0	42	0.7	.25
ApoE3/E2	8	16.3	708	11.4	.46
ApoE3/E3	28	57.1	4126	66.3	.26
ApoE4/E3	10	20.4	1155	18.5	.20
ApoE4/E4	2	4.1	77	1.2	.08
ApoE4/E2	0	0.0	122	2.0	--
[a]the data are from the study of Hubacek et al., 2013 [32].					
E2, E3, E4: different alleles of the gene coding apolipoprotein E. $p < .05$ was considered significant.					

The association of ApoE genotype with chronic structural and functional changes of the visual pathway of the patients is presented in the Table 4. The baseline genotype group was ApoE2/E2(E3/E2): as long as only one patient had E2/E2 genotype (see Table 3), he was added to the group of patients with E3/E2 genotype ($n = 8$). We compared the thickness of retinal nerve fiber layer (global one and in the temporal segments of retina) in three groups coded as "2" (genotypes E2/E2 + E3/E2), "3" (genotype E3/E3), and "4" (genotypes E4/E3 + E4/E4). Patients carrying the ApoE4 allele had lower global and temporal RNFL thickness at all three examinations compared to patients without the ApoE4 allele. The association between the genotype and the thickness of retina became even stronger with time (4 years after discharge compared to 6 months after discharge) what meant higher rate of RNFL decrease in the patients with E4/E3(E4/E4) genotype. The degree of acute demyelination of optic nerve presented by the latency P1 prolongation measured 6 months after discharge demonstrated significant association with ApoE genotype, but the process of remyelination led to the conductivity restoration and the loss of significance of this association with time.

Table 4. The association of ApoE genotype group (ApoE2/E2(E3/E2) versus ApoE3/E3 versus ApoE4/E3(E4/E4)) with chronic structural and functional changes of the visual pathway during the observation period ($n = 41$).Table Layout

Parameter	Mean value ± SD	R	*p*
RNFL OD global, 1st exam, mcm	88.3 ± 5.5	−0.418	**.007**
RNFL OD global, 2nd exam, mcm	84.6 ± 6.9	−0.466	**.002**
RNFL OD global, 3rd exam, mcm	83.3 ± 7.1	−0.458	**.003**
RNFL OS global, 1st exam, mcm	84.9 ± 6.4	−0.430	**.006**
RNFL: retinal nerve fibers layer thickness; OD: oculus dexter; OS: oculus sinister; exam: clinical examinations 6 months, 24 months, and 48 months after discharge			
$p < .05$ (bold figures) was considered significant.			

Parameter	Mean value ± SD	R	*p*
RNFL OS global, 2nd exam, mcm	81.9 ± 7.6	−0.463	**.002**
RNFL OS global, 3rd exam, mcm	80.4 ± 7.7	−0.455	**.003**
RNFL OD temporal, 1st exam, mcm	59.9 ± 5.3	−0.421	**.006**
RNFL OD temporal, 2nd exam, mcm	57.7 ± 6.0	−0.457	**.003**
RNFL OD temporal, 3rd exam, mcm	56.8 ± 5.9	−0.470	**.002**
RNFL OS temporal, 1st exam, mcm	55.1 ± 4.9	−0.353	**.03**
RNFL OS temporal, 2nd exam, mcm	53.6 ± 5.7	−0.377	**.02**
RNFL OS temporal, 3rd exam, mcm	53.6 ± 5.8	−0.387	**.01**
Latency P1 OD, 1st exam, ms	115.2 ± 2.2	0.362	**.03**
Latency P1 OS, 1st exam, ms	117.8 ± 3.5	0.373	**.02**
Latency P1 OD, 2nd exam, ms	98.3 ± 2.6	0.409	**.02**
Latency P1 OS, 2nd exam, ms	103.3 ± 5.3	0.303	.07
Latency P1 OD, 3rd exam, ms	99.3 ± 2.6	−0.141	.41
Latency P1 OS, 3rd exam, ms	101.8 ± 4.1	−0.034	.84
RNFL: retinal nerve fibers layer thickness; OD: oculus dexter; OS: oculus sinister; exam: clinical examinations 6 months, 24 months, and 48 months after discharge			
$p < .05$ (bold figures) was considered significant.			

To demonstrate that both for retinal nerve fiber layer thickness and for the latency P1 prolongation, as well as for the dynamics of further RNFL decrease the presence of E4 allele was the key factor, we divided the population on two groups, ApoE4 carriers (genotypes E4/E3 and E4/E4) versus non-carriers. Table 5 demonstrates the results of *t*-test between these two groups. The results of *t*-test confirmed the data on the effect of E4 allele on both morphological state of retina after methanol poisoning and on the function of visual pathway. Of five patients with non-elicitable VEP in at least one eye, four patients carried the ApoE4 allele. In the study population of methanol poisoning survivors, the odds ratio (95% CI) for abnormal VEP finding at the first examination (prolonged P1 latency and/or abnormal N1P1 amplitude or non-elicitable evoked potential) was 8.92 (3.00–36.50) for ApoE4 allele carriers compared to the non-carriers ($p < .001$).

Table 5. Apolipoprotein E genotype group (ApoE4 carriers versus non-carriers) and chronic structural and functional changes of the visual pathway during the observation period ($n = 41$).Table Layout

	ApoE4 carriers ($n = 11$)	ApoE4 non-carriers ($n = 30$)	*p*
RNFL OD global, 1st exam, mcm	76.0 ± 16.0	92.4 ± 5.0	.05
RNFL OD global, 2nd exam, mcm	68.0 ± 20.0	90.6 ± 5.7	**.03**
RNFL: retinal nerve fibers layer thickness; OD: oculus dexter; OS: oculus sinister; exam: clinical examinations 6 months, 24 months, and 48 months after discharge			
$p < .05$ (bold figures) was considered significant.			

	ApoE4 carriers ($n = 11$)	ApoE4 non-carriers ($n = 30$)	p
RNFL OD global, 3rd exam, mcm	65.0 ± 20.0	89.6 ± 6.0	.03
RNFL OS global, 1st exam, mcm	72.0 ± 17.0	89.3 ± 6.1	.07
RNFL OS global, 2nd exam, mcm	64.0 ± 20.0	88.4 ± 6.8	.03
RNFL OS global, 3rd exam, mcm	62.0 ± 20.0	86.8 ± 7.2	.03
RNFL OD temporal, 1st exam, mcm	46.0 ± 11.0	64.2 ± 5.5	.003
RNFL OD temporal, 2nd exam, mcm	42.0 ± 12.0	63.3 ± 6.1	.001
RNFL OD temporal, 3rd exam, mcm	40.0 ± 12.0	62.5 ± 5.9	.001
RNFL OS temporal, 1st exam, mcm	43.6 ± 8.6	58.9 ± 5.4	.006
RNFL OS temporal, 2nd exam, mcm	40.0 ± 11.0	58.5 ± 6.2	.003
RNFL OS temporal, 3rd exam, mcm	39.0 ± 10.0	58.8 ± 6.3	.000
Latency P1 OD, 1st exam, ms	120.1 ± 3.7	114.0 ± 2.4	.009
Latency P1 OD, 2nd exam, ms	103.8 ± 5.6	97.1 ± 2.9	.05
Latency P1 OD, 3rd exam, ms	89.0 ± 30.0	98.7 ± 3.2	.50
Latency P1 OS, 1st exam, ms	124.6 ± 9.8	115.7 ± 3.5	.033
Latency P1 OS, 2nd exam, ms	109.4 ± 9.5	101.8 ± 6.3	.16
Latency P1 OS, 3rd exam, ms	94.0 ± 32.0	100.5 ± 4.8	.66
RNFL: retinal nerve fibers layer thickness; OD: oculus dexter; OS: oculus sinister; exam: clinical examinations 6 months, 24 months, and 48 months after discharge			
$p < .05$ (bold figures) was considered significant.			

The presence of the ApoE4 allele was further associated with brain necrotic lesions on MRI ($r = 0.384$; $p = .01$) and brain hemorrhages due to methanol poisoning ($r = 0.395$; $p = .01$). No association of ApoE alleles with sex, age, acute methanol, formic acid or ethanol serum concentration, as well as the treatment modalities (antidote, mode of hemodialysis, folate substitution) was observed.

Discussion

The prevalence of visual sequelae of acute methanol poisoning is high but typically underestimated. In our previous studies, we demonstrated that the population of patients with long-term visual damage after methanol-induced toxic optic neuropathy comprised up to 40% of the survivors, and acute retinal ganglion cells injury was followed by chronic retinal neurodegeneration and progressive axonal loss occurred in up to 25% of the patients. [18,35]. In the present study, we found that total or partial functional restoration of optic nerve conductivity after acute myelin sheath damage occurred in more than 80% of patients during 4 years, with the highest rate of remyelination within 2 years of discharge. The time between methanol exposure and hospital admission, severity of acidemia, and patient age were the most significant variables for the dynamics of optic nerve conductivity restoration. The VEP amplitude, abnormal in one fourth of the study population at discharge from hospital, demonstrated the tendency of this variable to progressively

decrease during the following years. In half of the patients with measurable evoked potential, a decrease of N1P1 amplitude of more than 1.0 mcV was observed; none of these patients had abnormal amplitude at discharge from the hospital. In 2–7% of examined eyes, the progression of visual pathway changes led to worsening function, from abnormal, but still measurable N1P1 amplitude, to a non-elicitable VEP. The effect of the type of antidote administered in hospital and of folate substitution therapy was not significant in our study; however, patients treated with intermittent hemodialysis compared to the continuous modalities demonstrated better functional outcome: shorter P1 latency and higher N1P1 amplitude of the VEP. Finally, we demonstrated the association between the ApoE genotype polymorphism and chronic structural and functional changes of the visual pathway. Patients who carried the *ApoE4* allele had reduced RNFL thickness and longer P1 latency compared to non-carriers. *ApoE4* presence was associated with necrotic lesions and hemorrhages on MRI of the brain.

Acute methanol poisoning is a life-threatening condition with a lethality rate of up to 30–40%, and necrotic basal ganglia damage occurs in up to 50% of survivors [36–39]. However, long-term visual sequelae due to optical nerve and retina damage by formic acid should not be underestimated and underdiagnosed due to their impact on the quality of life of the patients and their relatives, as well as on the medical facilities providing health care after discharge from the hospital [17–19,40]. Optic nerve axons are the most vulnerable structures for acute neurotoxic effects of formic acid; nevertheless, chronic functional changes of the visual pathway in patients after acute exposure to methanol have not yet been well studied. One reason is that typically after discharge from hospital contact with the patients is lost [19]. In our previous studies, we demonstrated that structural changes of the ocular retina, namely chronic decrease of RNFL, may progress in the years following discharge from hospital [35,41]. These changes may affect visual pathway functions and may be registered by the series of VEP measurements within the longitudinal follow-up study.

In the case reports and small retrospective case series studies, both partial recovery and progression of visual loss were registered typically 6–9 months after discharge [17,19,42]. We reported that the process of chronic optic nerve remyelination in patients with mild to moderate damage of myelin sheaths may lead to improvement of conductivity in survivors without a significant increase of VEP amplitude [43,44]. Our present study demonstrates that the process of chronic remyelination leads to the restoration of optic nerve conductivity in most survivors, with the highest rate of remyelination during the first 2 years after discharge. Patients without positive dynamics of optic nerve conductivity or with worsening visual pathway function exhibited the most severe initial neuronal damage with non-elicitable evoked potential at discharge or initial P1 latency longer than 120 ms. These patients typically had severe acidemia, high serum formate, lactate, and no protective ethanol in blood serum on admission to the hospital.

Severe acidemia is one of the prognostic factors of mortality in acute methanol poisoning [45,46]. In our study, the degree of acidemia on admission was associated with the dynamics of P1 latency and provided the basis for the model prediction of optic nerve conductivity restoration during the observation period. This model accurately predicted the actual data on remyelination dynamics for most of the patients in our study population. Undissociated formic acid, but not the formate anion, easily crosses the blood-brain barrier and neuronal cell membranes [47]. The dissociation constant for formic acid is 3.8; therefore, for this weak acid, a pH drop of 0.3 would mean a doubling of undissociated formic acid levels, and hence a significant increase in ocular neurotoxicity [10–12]. This fact may explain the association between the modality of dialysis and both P1 latency and N1P1 amplitude of VEP. Higher rates of formate elimination and acidemia correction during intermittent hemodialysis compared to the continuous modalities may have an impact on the toxic effects of formate on optical neurons [28,29,48].

Our findings suggest that no restoration of the VEP amplitude occurs during the years following acute neuronal damage. During the observation period, the mean amplitude did not significantly change in the study population. The character of association between the VEP N1P1 amplitude and P1 latency, with an observed gradual loss of a significant correlation between these parameters during 4 years after discharge, reflected the process of conductivity restoration of the optic nerve due to remyelination without corresponding amplitude restoration after methanol-induced toxic neuropathy. Nevertheless, a further decrease of the amplitude, probably due to chronic axonal degeneration, was observed in almost half of the patients with measurable N1P1 amplitude during the observation period. The mean amplitude decrease in this subgroup was significantly higher than in the total study population, with the highest rate of up to 5–7 mcV over the 4 years. An abnormal N1P1 amplitude was measured in 24–33% of eyes at the third examination, a finding that reflected the proportion of patients with diagnosed chronic retinal neurodegeneration reported in our previous study [35]. Therefore, progressive chronic axonal loss leads to a decrease in N1P1 amplitude over the years following acute methanol poisoning. It should be emphasized that the decrease in VEP amplitude was registered in patients with initially normal N1P1 values measured at the first examination.

Other conditions unrelated to methanol exposure may affect optic nerve conductivity and functions of the visual pathway in the patients from our study population. Vitamin B_{12} or B_1 deficiency caused by chronic alcohol abuse or nutritional deficits, thyroid gland hypofunction, or insufficiently controlled glycemia in diabetes mellitus may negatively impact remyelination after methanol-induced optic neuropathy and may cause axonal damage due to inadequate restoration of myelin sheaths trophic and protective functions [49–53]. However, in our study, no association was found between repeatedly measured concentrations of serum glucose, TSH, vitamins B_{12} or B_1, and the dynamics of chronic visual pathway functional changes. Nevertheless, conductivity restoration was relatively slower in patients with high GGT liver enzyme, which is one of the clinical signs of chronic alcohol abuse.

In our previous studies, we demonstrated that genetic polymorphisms of the enzymes involved in methanol oxidation affects the outcome of poisoning [54,55]. In the present study, we focused on the ApoE polymorphism that is not directly involved in methanol metabolism but plays an important role in eye development and metabolism. ApoE, as a pleiotropic protein that influences the risk of Alzheimer's and cardiovascular diseases, attracts attention as a potential determinant of the visual functions. This glycoprotein is synthetized in the retina by Muller glial cells and transported into the optic nerve by retinal ganglion cells, where it plays a key role in the local transport and distribution of cholesteryl ester-rich lipoproteins to supply the optic axons' need for particular lipids, since apolipoprotein B, the other molecule with the same function, is not synthetized in the CNS [56,57]. ApoE produced by Muller glial cells donates lipids and cholesterol to the neurons engaged in synaptic remodeling following acute lesioning [56].

The lipid transport function of E4 form of ApoE is worse compared to the other two isoforms of ApoE, as experimental studies indicate [58]. Further, *ApoE4* allele has been linked to impaired blood-brain barrier function, what may reflect a breakdown in the blood-retina barrier and higher exposure of retinal ganglion cells and optic nerve axons to the toxic metabolites of methanol [59,60]. Finally, in optic neurons, ApoE4 reduces expression of the protein subunits of mitochondrial respiratory complexes, such as subunit 1 of complex IV (mtCOX1) and subunit of complex V, resulting in a reduction in mitochondrial respiratory function [61]. Similarly, ApoE4 perturbs neuronal function by reducing mitochondrial motility, decreasing neurite outgrowth, and inhibiting synaptogenesis [62–64]. Therefore, patients with the E4 isoform may be more susceptible to the deleterious effects of formic acid on retinal ganglion cells and their axons.

ApoE genotype distribution was similar in our patients to the general Czech population [32]. We found an association between ApoE polymorphism and chronic visual pathway changes in the survivors of methanol poisoning. ApoE4 is associated with both neuronal and vascular impairment of the retina. In experimental studies, the ApoE4 genotype was associated with retinal vascular pathology, reduced levels of vascular endothelial growth factor (VEGF), and a significant decrease in retinal synaptic density in developing mice retina [65]. The synaptic density of the retinal neuronal synaptic layers was significantly lower in ApoE4 compared to ApoE3 mice, with reduced levels of the presynaptic vesicular glutamatergic transporter that suggested glutamatergic nerve terminals are preferentially affected in ApoE4 allele carriers [66].

Our observation suggests that ApoE4 might be associated with chronic retinal neurodegeneration and progressive visual pathway dysfunction. Further, the association among ApoE polymorphism, chronic retinal neuronal degeneration, and MRI findings of brain hemorrhages and necrotic damage in patients exposed to methanol indicates possible pathophysiological relationships between long-term visual and brain sequelae of poisoning. Our study demonstrates that the signs of progressive chronic visual pathway dysfunction may indicate association with a genetic risk factor for chronic neurodegenerative diseases in the population of survivors of acute methanol poisoning.

Limitations of the study

There were several limitations of this study. The limited number of the patients with methanol-induced toxic optic neuropathy could have an effect on the power of the study. The study was not controlled for the time to hospital admission, treatment, and comorbidities of the patients hospitalized with acute methanol poisoning who were included in the study. However, the patients in the study population were relatively young; the number of patients with comorbidities was low, and the clinical and laboratory parameters monitored during the observation period provided us with sufficient evidence to exclude these comorbidities as possible causes of chronic visual pathway functional changes.

The aim of the study was to register the dynamics of visual pathway function after acute methanol-induced toxic optic neuropathy in poisoning survivors during 4 years after discharge, to compare this data with data from controls of

the same age and alcohol abuse profile, and to find any association between the dynamics of optical functions and the clinical and laboratory parameters followed during hospitalization and after discharge. Approximately one third of methanol poisoning survivors did not join the follow-up study; therefore, selection bias is possible, with fewer severely poisoned subjects participating in follow-up. However, the admission laboratory data demonstrated that at least half of the patients were severely poisoned and reported at admission subjective signs of visual impairment.

Despite these limitations, this study represents the first prospective longitudinal cohort study of the dynamics and determinants of chronic visual pathway functional changes after methanol-induced optic neuropathy performed during 4 years after discharge by applying a standardized clinical examination protocol and advanced technological measurements in the same medical facility.

Disclosure statement

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper. The manuscript has been read and approved by all authors.

The authors certify that the submission (aside from an abstract) is not under review at any other publication.

The authors certify that the authors have no other submissions and previous reports that might be regarded as overlapping with the current work.

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11.6. PŘÍLOHA VI

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Use of out-of-hospital ethanol administration to improve outcome
in mass methanol outbreaks.

Annals of Emergency Medicine, 2016**; 68(1):** 52-61
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TOXICOLOGY/ORIGINAL RESEARCH

Use of Out-of-Hospital Ethanol Administration to Improve Outcome in Mass Methanol Outbreaks

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Study objective: Methanol poisoning outbreaks are a global public health issue, with delayed treatment causing poor outcomes. Out-of-hospital ethanol administration may improve outcome, but the difficulty of conducting research in outbreaks has meant that its effects have never been assessed. We study the effect of out-of-hospital ethanol in patients treated during a methanol outbreak in the Czech Republic between 2012 and 2014.

Methods: This was an observational case-series study of 100 hospitalized patients with confirmed methanol poisoning. Out-of-hospital ethanol as a "first aid antidote" was administered by paramedic or medical staff before the confirmation of diagnosis to 30 patients; 70 patients did not receive out-of-hospital ethanol from the staff (12 patients self-administered ethanol shortly before presentation).

Results: The state of consciousness at first contact with paramedic or medical staff, delay to admission, and serum methanol concentration were similar among groups. The median serum ethanol level on admission in the patients with out-of-hospital administration by paramedic or medical staff was 84.3 mg/dL (interquartile range 32.7 to 129.5 mg/dL). No patients with positive serum ethanol level on admission died compared with 21 with negative serum ethanol level (0% versus 36.2%). Patients receiving out-of-hospital ethanol survived without visual and central nervous system sequelae more often than those not receiving it (90.5% versus 19.0%). A positive association was present between out-of-hospital ethanol administration by paramedic or medical staff, serum ethanol concentration on admission, and both total survival and survival without sequelae of poisoning.

Conclusion: We found a positive association between out-of-hospital ethanol administration and improved clinical outcome. During mass methanol outbreaks, conscious adults with suspected poisoning should be considered for administration of out-of-hospital ethanol to reduce morbidity and mortality. [Ann Emerg Med. 2016;■:1-10.]

Please see page XX for the Editor's Capsule Summary of this article.

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INTRODUCTION

Background

Mass methanol poisonings represent a challenge for health care providers throughout the world because of the distillation and consumption of illicit alcohol.[1-5] Morbidity and mortality in methanol poisoning remain high; timely diagnosis is difficult, and the onset of treatment is often delayed.[6-8] During 2000 to 2012, more than 50 mass methanol outbreaks with approximately 5,000 poisoned subjects and more than 2,000 fatalities occurred worldwide.[9] If specific interventions are inadequate or delayed, mortality exceeding 40%, permanent visual impairment, and motor and cognitive disorders may occur.[10-12]

Although mass or cluster methanol poisonings occur regularly, especially in developing countries, reports of larger outbreaks in which complete admission clinical and laboratory data, medical treatment protocols, and outcomes are accurately documented and analyzed are scarce.[1,2] During the Czech Republic methanol poisoning outbreak in 2012 to 2014, there was a unique opportunity to study a mass exposure because sufficient medical and public health infrastructure allowed comprehensive data collection and evaluation, as well as a coordinated out-of-hospital intervention within the national health care system.

Methanol is not toxic itself, but it is metabolized to the highly toxic formic acid/formate ion, which inhibits mitochondrial respiration.[13-16] The accumulation of formic acid may result in metabolic acidosis, visual impairment, and damage of the basal ganglia, especially when its concentration increases above 36 to 46 mg/dL.[17-20] Rapid administration of antidotes (such as fomepizole or ethanol) that prevent toxic metabolite formation by blocking the

Editor's Capsule Summary

What is already known on this topic

Delayed treatment with an antidote is known to worsen the outcome of methanol poisoning.

What question this study addressed

Does the out-of-hospital administration of ethanol decrease mortality and morbidity of methanol poisoning?

What this study adds to our knowledge

In this case series of 100 methanol overdoses, the 30 patients who received out-of-hospital ethanol had improved survival and fewer visual and central nervous system deficits than those who did not.

How this is relevant to clinical practice

Although this study was uncontrolled, it provides support for the out-of-hospital administration of ethanol in mass-casualty methanol overdose events.

alcohol dehydrogenase enzyme is crucial for successful treatment.[21-23]

Importance

The role of ethanol in the treatment of acute methanol poisoning is well established.[24-26] Ethanol has approximately 10 times higher affinity for alcohol dehydrogenase than methanol, and a serum concentration of 100 to 150 mg/dL is sufficient to completely block the metabolism of methanol to formate in methanol concentrations that most poisoned patients have on admission.[27] The indications for hospital ethanol administration are a documented plasma methanol concentration of more than 20 mg/dL, a high osmolal gap with documented recent history of ingesting toxic amounts of methanol, or a metabolic acidosis with history or strong clinical suspicion of poisoning.[14]

Because of the high morbidity and mortality of methanol poisoning, ethanol should be administered as soon as possible after methanol ingestion.[14,24] Its wide availability in the community compared with fomepizole makes it attractive for an out-of-hospital "first aid" approach. Out-of-hospital administration of ethanol by paramedics or medical staff as an antidote in methanol outbreaks has previously been tried,[2] but to our knowledge the safety and effectiveness of this approach has not been assessed.

Goals of This Investigation

Close collaboration between the Ministry of Health, Czech Republic, the Toxicological Information Center, and national hospitals allowed us to address this question during a recent methanol mass poisoning in the Czech Republic.[28] We aimed to evaluate the association between out-of-hospital ethanol administration and outcome in patients with a high suspicion of methanol poisoning before laboratory confirmation could be obtained.

MATERIALS AND METHODS

Study Design

This was a prospective, observational, case-series study of patients with acute methanol poisoning treated in hospitals during the Czech Republic mass methanol poisoning outbreak from September 3, 2012, until August 31, 2014. The admission data, including out-of-hospital treatment, were collected prospectively by the treating providers, using a standardized data collection form (Appendix E1, available online at http://www.annemergmed.com) and sent to the Toxicological Information Center on the day after each admission to the hospital. The data on hospital treatment and outcome were collected and reviewed retrospectively from the hospital discharge reports. The study was approved by the General University Hospital Ethics Committee in Prague, Czech Republic.

Setting

The study was conducted in 30 hospitals in 11 regions of the Czech Republic, where the poisoned patients were treated. These hospitals were located in the regional city centers, had ICUs and toxicologic laboratories, and were equipped with hemodialysis and gas chromatography facilities. The patients were transferred to the regional hospitals by emergency medical services (EMS) ambulance or self-presented. EMS is a national system in the Czech Republic and is staffed with physicians and advanced life support providers.

The medical facilities situated in smaller localities were the first presentation points ("collecting points") for the patients from these localities. These hospitals were able to provide the physical examination, breath alcohol test, and osmolality measurement by freezing point depression, but could not confirm the methanol concentration and could not provide dialysis or intensive care. Patients with suspicion of acute methanol poisoning from collecting points were transferred by ambulance to the secondary regional hospitals.

Selection of Participants

All patients hospitalized with confirmed acute methanol poisoning were eligible for this study (Figure). Patients

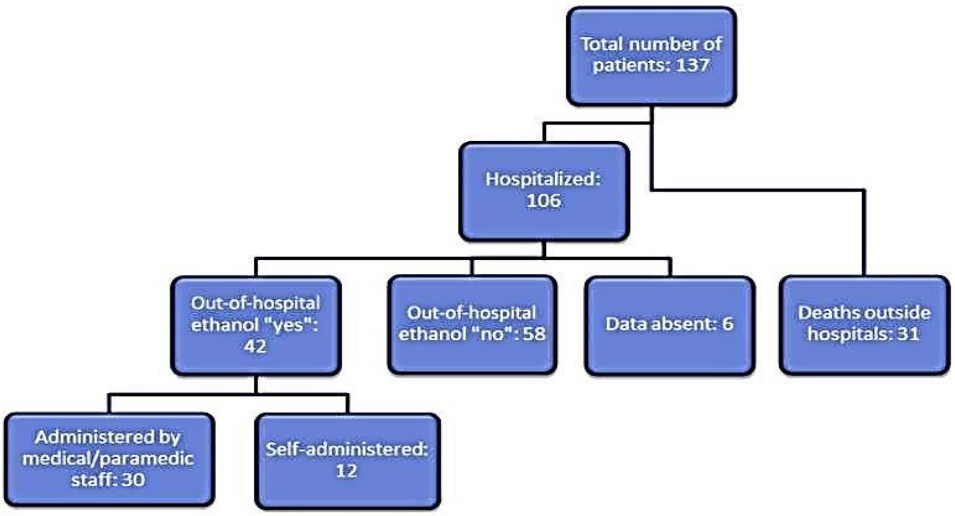

Figure. Flowchart of the study design.

were excluded if they died out of the hospital, if their data on out-of-hospital ethanol administration could not be obtained, or if blood samples for serum ethanol measurement were not taken before hospital treatment with ethanol.

To identify the cases, mandatory reporting to the Ministry of Health and the Czech Republic Toxicological Information Center on all cases of hospital admission with laboratory-confirmed methanol poisoning and nationwide daily monitoring of the situation in all hospitals started on September 6, 2012, 3 days after admission of the first 3 patients with acute methanol poisoning.

Interventions

A recommendation to administer out-of-hospital ethanol to all patients with suspected methanol poisoning was made by the Toxicological Information Center and distributed to all medical facilities nationwide 2 weeks into the epidemic, when the ambulance and emergency department (ED) staff had become more alert to potential methanol poisoning cases. The patients treated before hospitalization received oral ethanol either directly from ambulance crews or at local collecting points before transfer to a higher-level hospital.

The protocol of out-of-hospital ethanol administration was predominantly applied in fully conscious patients with strong clinical suspicion of methanol poisoning before admission to the higher-level hospital and definite diagnosis of poisoning. The recommended oral loading dose of ethanol was 1.8 to 2.0 mL/kg body weight of 40% alcohol by volume of ethanol, with the aim of achieving serum ethanol concentrations of at least 100 mg/dL.[14] Postadmission treatment was similar in the 2 groups in regard to ethanol treatment, folate substitution, and elimination techniques. The data on postadmission treatment were reviewed from the hospital records sent to the Toxicological Information Center within the mandatory reporting system.

Methods of Measurement

A modified standardized form for collection of admission data based on a methanol outbreak in Norway in 2002 to 2004[1] was distributed to all hospitals during the second week of the outbreak and used for the prospective chart review (Appendix E1, available online at http://www.annemergmed.com). The heads of the EDs of 30 regional hospitals where poisoned patients were admitted were instructed by the research coordinators by telephone interviews and e-mails about the procedure of filling out the forms, primary data collection techniques, and training and supervising of the abstractors. The emergency physicians who admitted and examined the patients collected the primary demographic, anamnestic, clinical, biochemical, and toxicologic data and completed the standardized forms as part of their mandatory task of daily reporting of new cases of methanol poisoning. Both the abstractors and the heads of the EDs were blinded to the study hypothesis of the effectiveness and safety of out-of-hospital ethanol administration. A detailed history of the poisoning and of ocular and systemic toxicity was obtained

directly from the patient or from relatives of critically ill patients on admission to the hospital. The completed standardized admission data collection forms were sent by the ED to the Toxicological Information Center by e-mail or fax the day after admission, when the results of toxicologic assay confirmed the diagnosis of methanol poisoning. The Toxicological Information Center provided immediate feedback for the form's completeness. The data on the patients admitted before distribution of the protocol were collected retrospectively.

The discharge reports of all hospitalized patients with a confirmed diagnosis containing the results of neurologic and ophthalmologic examinations on admission, during hospitalization, and on discharge and the detailed report on the postadmission hospital treatment, results of biochemical and toxicologic monitoring, adverse reactions, and complications of treatment were collected retrospectively and analyzed in the Toxicological Information Center.

Laboratory analyses were performed on admission. Diagnosis was established when a history of recent ingestion of illicit spirits was available and serum methanol was higher than 20 mg/dL, or when there was a history or clinical suspicion of methanol poisoning and serum methanol was above the limit of detection, with at least 2 of the following: pH less than 7.3, serum bicarbonate level less than 20 mEq/L, or anion gap greater than or equal to 20 mEq/L.

The clinical examination protocol included complete ocular examination with standard ophthalmologic tests (visual acuity, color vision, visual fields, contrast sensitivity, and fundus examination), cerebral computed tomography (CT) or magnetic resonance imaging (MRI) of the brain, and standard neurologic examination (including the Mini-Mental State Examination,[29] motor, sensory, cerebellar, cranial nerves, and reflexes). Patients were considered to have visual sequelae of acute methanol poisoning if the symptoms of toxic neuropathy of the optic nerve were documented on admission or during hospitalization, with pathologic findings on visual acuity, visual fields, color vision, contrast sensitivity, and persisting lesions on fundoscopy. Similarly, patients were considered to have central nervous system sequelae of poisoning if symmetric necrosis and hemorrhages of basal ganglia were present on CT or MRI.

The hospitalized patients were retrospectively assigned to 3 groups defined according to outcome: group 1, patients who survived without sequelae; group 2, patients who survived with visual or central nervous system sequelae; and group 3, patients who died. These groups were each further divided into 2 subgroups: "with out-of-hospital ethanol administration by EMS staff (ethanol EMS positive)" and "without out-of-hospital ethanol administration by EMS staff (ethanol EMS negative)." Within the latter subgroup, data from patients who self-administered ethanol shortly before presentation were analyzed separately.

Outcome Measures

The primary outcome of this study was mortality in the groups of patients with and without out-of-hospital ethanol administration. The secondary outcome was the number of survivors with visual sequelae and central nervous system sequelae of poisoning at discharge from hospitals.

Primary Data Analysis

The number of subjects with missing key data on out-of-hospital ethanol administration and serum ethanol concentration on admission before any hospital treatment was low (5.7%). We chose to exclude these subjects because no multivariate logistic regression model testing the study hypothesis with imputed values was applied as a result of the sample size.

To test the strength and the direction of association between out-of-hospital ethanol administration, positive serum ethanol on admission, and the outcome of treatment, we used both the total study population and the population of hospitalized patients after exclusion of those with a Glasgow Coma Scale (GCS) score of 10 points or fewer at presentation (contraindication for out-of-hospital ethanol administration).

As long as there was a risk of conflicting data with false-positive results (eg, on the basis of interview of ambulance staff, out-of-hospital ethanol was administered according to the recommendation) or false-negative results (eg, on the basis of interview, no ethanol was self-administered by the patient) caused by misrepresentation of history, the coding of conflicting data was suggested. However, we registered no false-positive or false-negative cases: positive serum concentration of ethanol was detected analytically in the cases of out-of-hospital ethanol administration, and no patients with negative history had a positive serum ethanol result on admission.

Descriptive statistics were assessed with medians with interquartile ranges, Spearman's rank correlation, exploratory factor analysis, and χ^2 tests. Statistical documentation was performed in Microsoft Excel 2010 (Redmond, WA), and the formal calculations were produced in QC Expert software (version 3.1; Trilobyte, Pardubice, Czech Republic) and in SPSS (version 17.0; SPSS Inc., Chicago, IL).

RESULTS

Of 137 patients, 31 (22.6%) died before contact with paramedic or medical staff and presentation to the hospital (Figure). Of the remaining 106 patients, data on out-of-hospital ethanol administration could not be obtained, or, for 6 patients, blood samples for serum ethanol measurement were not taken before hospital antidote treatment with ethanol. Of the 100 patients included, 61 were transferred to the hospital by ambulance. The remaining 39 were self-presenters, who visited the ED personally, were transported to the hospitals by their relatives, or were transported to the hospitals by police (10 cases).

Thirty patients (30%) received out-of-hospital ethanol either directly from ambulance crews (15/30) or from medical or paramedical staff at the collecting points (15/30) (ethanol EMS–positive patients). The estimated time range to secondary hospital admission from presentation to a collecting point was 1.5 to 3.5 hours.

The remaining 70 patients did not receive out-of-hospital ethanol from paramedical or medical staff (ethanol EMS–negative patients). Among them, 12 patients self-administered an unknown amount of ethanol before first contact because they believed the symptoms represented a hangover. Five of these patients were then transferred to the hospital by ambulance, 5 self-presented, and 2 were transported by the police. Fifty-eight patients did not receive ethanol before admission to the hospital. Most of them (41/58) were transferred to the hospitals by ambulance, whereas the rest self-presented.

Seventy eight patients were awake, with GCS score greater than 10, whereas 22 patients had a GCS score of 10 points or fewer on initial presentation. In patients with a GCS score greater than 10 points, out-of-hospital ethanol was administered by paramedic or medical staff to 27 of 78 patients (34.6%) and not administered to 51 of 78 (65.4%), whereas for patients with a GCS score of 10 points or fewer, it was administered in 3 of 22 (13.6%). Among the 100 patients, only 12% were admitted within 12 hours of methanol ingestion, 61% within 13 to 48 hours, and 13% after 48 hours (14% unknown).

The median serum ethanol level on admission in 30 patients with out-of-hospital administration by paramedic or medical staff (Table 1) was 84.3 mg/dL (interquartile range 32.7 to 129.5 mg/dL) and 141.0 mg/dL (interquartile range 29.5 to 377.4 mg/dL) in 12 patients who self-administered. The serum methanol concentration on admission in ethanol EMS–positive patients was similar to that of ethanol EMS-negative ones. The ethanol EMS–positive patients were less acidotic on admission, with higher arterial blood pH and lower base deficit, anion gap, and serum formate and lactate concentrations (Table 1).

Common clinical features included visual and gastrointestinal disturbances, dyspnea, chest pain, and coma (Table 2). Other less common features included fatigue, headache, dizziness, somnolence, anxiety, alcoholic delirium, tremor, seizures, and cardiac and respiratory arrest. The median ethanol concentration was higher in patients without clinical symptoms (50.2 mg/dL [range 5.1 to 137.7 mg/dL]) than in those with clinical features (0 mg/dL [range 0 to 23.0 mg/dL]).

Thirty patients (30%) received ethanol from paramedic or medical staff before presentation to a hospital able to provide definitive care. The alertness of staff for methanol poisoning increased with time, when typical symptoms were increasingly likely to have been caused by methanol poisoning. In regard to symptoms, 80% of the patients who received out-of-hospital ethanol from paramedic or medical staff had a history of suspected methanol ingestion plus at least 1 other clinical finding (including signs of inebriety); 30% had visual symptoms (blurry or cloudy vision, central visual field defects, and alterations in light, color, and depth perception, progressing to total blindness with absent direct pupillary response) or dyspnea. The other 20% had a history of drinking methanol with patients who had already been hospitalized for methanol poisoning.

The state of consciousness was a limiting factor for providing out-of-hospital ethanol. In general, ethanol was administered to patients who were less sick (awake, with GCS score >10) and was not administered to those who were unconscious. Of 78 patients with GCS score greater than 10 points at presentation, 27 (34.6%) received ethanol from paramedic or medical staff; in 12 cases (15.4%), ethanol was self-administered by the patient. In 39 patients with GCS score greater than 10 points at presentation, out-of-hospital ethanol was not administered; most of them (28 of 39) were hospitalized during the first 2 weeks of the outbreak.

Twenty two patients had a GCS score of 10 or fewer points on the arrival of the ambulance. Three of these patients received out-of-hospital ethanol. One of these 3 patients developed coma and was severely acidotic, with a serum methanol level of 348.0 mg/dL and serum ethanol level of 23.0 mg/dL. No other cases of coma or other adverse effects of the treatment protocol were recorded in the study population. Detailed information about the postadmission treatment in hospitals is presented in Table 3.

Overall, patients receiving out-of-hospital ethanol from paramedic or medical staff or by self-administration showed

Table 1. Laboratory data on admission for 100 hospitalized patients, according to the outcome groups.*

			Group 1 (n=49)		Group 2 (n=30)		Group 3 (n=21)		
Characteristic	**EtOH EMS–Positive (n=30)**	**EtOH EMS–Negative (n=70)**	**EtOH EMS–Positive (n=27)**	**EtOH EMS–Negative (n=22)**	**EtOH EMS–Positive (n=3)**	**EtOH EMS–Negative (n=27)**	**EtOH EMS–Positive (n=0)**	**EtOH EMS–Negative (n=21)**	**Total (n=100)**
Age (IQR), y	55 (47–64)	52 (37–60)	54 (47–62)	52 (35–58)	65 (56–69)	48 (37–58)	—	58 (45–63)	54 (38–61)
Serum methanol, mg/dL (IQR)	59.6 (29.2–138.1)	93.9 (41.7–180.4)	50.0 (29.5–133.0)	68.6 (39.4–101.9)	99.0 (58.0–223.7)	162.2 (80.1–263.1)	—	109.3 (69.2–189.1)	92.0 (39.4–176.0)
Serum ethanol, mg/dL (IQR)	84.3 (32.7–129.5)	0 (0–0)	88.9 (42.9–137.3)	7.4 (0–115.2)	23.0 (16.6–51.6)	0 (0–0)	—	0 (0–0)	0 (0–58.5)
Serum formate, mg/dL (IQR)	31.8 (5.5–59.8)	67.7 (53.8–76.9)	22.6 (4.6–52.0)	60.8 (36.4–70.4)	62.1 (62.1–62.1)	70.9 (62.1–85.1)	—	71.3 (58.9–73.6)	66.3 (41.0–76.4)
Serum lactate, mg/dL (IQR)	22.5 (17.1–32.4)	54.1 (17.1–83.8)	22.5 (17.1–30.6)	18.9 (15.3–36.0)	43.2 (28.8–56.8)	28.8 (12.6–66.7)	—	84.7 (60.4–116.2)	32.4 (17.1–70.3)
pH (IQR)	7.34 (7.20–7.42)	7.03 (6.79–7.26)	7.36 (7.25–7.42)	7.31 (7.25–7.41)	7.16 (7.01–7.18)	7.02 (6.83–7.17)	—	6.79 (6.65–6.93)	7.18 (6.89–7.34)
pCO_2, mm Hg (IQR)	33.8 (26.3–36.0)	30.0 (20.3–35.3)	34.5 (29.3–36.8)	32.3 (27.0–37.5)	19.5 (17.3–24.8)	21.8 (14.3–27.0)	—	33.8 (26.3–45.8)	30.8 (21.0–36.0)
HCO_3^-, mEq/L (IQR)	18.4 (11.6–22.6)	6.8 (4.1–13.5)	20.9 (12.8–22.8)	18.5 (8.8–22.7)	5.9 (4.7–8.7)	5.1 (3.6–9.3)	—	5.2 (3.9–7.7)	8.8 (4.7–19.5)
BE, mEq/L (IQR)	−6.1 (−1.5 to −14.6)	−23.2 (−11.3 to −29.0)	−3.6 (−1.2 to −12.8)	−4.5 (−1.7 to −15.6)	−22.1 (−19.6 to −27.5)	−25.4 (−19.1 to −27.5)	—	−29 (−26.9 to −31.9)	−17.8 (−3.7 to −27.7)
AG, mEq/L (IQR)	20.3 (18.3–28.6)	32.3 (22.3–39.8)	20 (18.1–26.8)	23.2 (18.2–28.5)	30.9 (29.8–31.9)	32.7 (25.3–37.7)	—	40.4 (34.8–45.1)	28.3 (19.4–36.3)
OG, mOsm/kg H_2O (IQR)	47 (21–73)	45.4 (23–77)	36 (22–73)	26 (19–44)	52 (33–86)	64 (39–100)	—	65 (45–136)	46.8 (21.7–75.9)
Serum glucose, mg/dL (IQR)	111.7 (102.7–136.9)	149.5 (111.7–234.2)	108.1 (102.7–129.7)	118.9 (109.9–147.7)	138.7 (127.9–183.8)	136.9 (108.1–203.6)	—	228.8 (185.6–290.1)	131.5 (108.1–201.8)
Time to treatment (IQR), h	25 (17–48)	48 (24–48)	26 (14–48)	24 (22–48)	24 (21–36)	48 (30–50)	—	48 (38–52)	41 (24–48)

EtOH, Ethanol; *IQR*, interquartile range; *BE*, base excess; *AG*, anion gap; *OG*, osmolal gap; *time to treatment*, time between toxic alcohol ingestion and start of hospital treatment.

To convert from mg/dL to mmol/L, use the following conversion factors: methanol 3.205; ethanol 4.608; formate 4.603; lactate 9.009; and glucose 18.018. To convert bicarbonate and base deficit from mEq/L to mmol/L, use the conversion factor 1.0. To convert mm Hg (torr) to kPa, use the conversion factor 7.501.

*Data are presented as medians with interquartile ranges. EtOH EMS–positive: patients with out-of-hospital ethanol administration by EMS (paramedic/medical staff); EtOH EMS–negative: patients without out-of-hospital ethanol administration by EMS (paramedic/medical staff; group 1, survivors without sequelae; group 2, survivors with sequelae; group 3, died.

Table 2. Clinical symptoms on admission in 100 hospitalized patients according to the outcome groups.*

Characteristic	Group 1 (n=49)		Group 2 (n=30)		Group 3 (n=21)	Total (n=100)	
	EtOH EMS–Positive (n=27)	EtOH EMS–Negative (n=22)	EtOH EMS–Positive (n=3)	EtOH EMS–Negative (n=27)	EtOH EMS–Negative (n=21)	EtOH EMS–Positive (n=30)	EtOH EMS–Negative (n=70)
No symptoms, No. (%)	16 (59)	6 (27)	1 (33)	0	0	17 (57)	6 (9)
Visual disturbances, No. (%)	4 (15)	10 (45)	3 (100)	8 (30)	12 (57)	7 (23)	30 (43)
Gastrointestinal disturbances, No. (%)	7 (26)	18 (82)	2 (67)	8 (30)	10 (48)	9 (30)	36 (51)
Dyspnea, No. (%)	1 (4)	10 (45)	1 (33)	8 (30)	11 (53)	2 (7)	29 (41)
Chest pain, No. (%)	1 (4)	1 (5)	0	1 (4)	7 (33)	1 (3)	9 (13)
Respiratory arrest, No. (%)	0	0	0	0	3 (14)	0	3 (4)
Coma, No. (%)	0	4 (18)	1 (33)	8 (30)	15 (71)	1 (3)	27 (39)

*EtOH EMS–positive: patients with out-of-hospital ethanol administration by EMS (paramedic/medical staff); EtOH EMS–negative: patients without out-of-hospital ethanol administration by EMS (paramedic/medical staff; group 1, survivors without sequelae; group 2, survivors with sequelae; group 3, died.

increased survival without sequelae and fewer deaths than those not receiving it (Tables 4 and 5). Among the 12 patients who self-administered ethanol before hospitalization, 11 (92%) survived without sequelae. One patient presented 36 hours after methanol ingestion with a serum ethanol level of 80.2 mg/dL and serum methanol level of 99.0 mg/dL and had visual sequelae on discharge. This patient had a serum lactate level of 65.8 mg/dL, serum formate level of 96.7 mg/dL, pH 7.0, and bicarbonate level of 10.2 mEq/L on admission, suggesting that he was severely poisoned before drinking ethanol.

All 27 patients with a GCS score greater than 10 who received out-of-hospital ethanol from medical staff survived without sequelae. Only 3 patients with a GCS score of 10 or fewer points received out-of-hospital ethanol; none died and 1 developed visual and central nervous system sequelae.

In contrast, in the presumably "less sick" patients with a GCS score greater than 10 who had not received out-of-hospital ethanol from paramedics or medical staff, only 22 of 57 (38.6%) survived without sequelae. Moreover, 11 of these 22 patients self-administered ethanol shortly before presentation. Therefore, only 11 of 46 patients (23.9%) less sick without ethanol from any source survived without sequelae.

LIMITATIONS

The limitations of this study include lack of randomization and confounding, leaving the possibility of inherent bias between the groups. Direct communication by telephone and e-mails with physicians who admitted and treated poisoned patients was applied to specify the key data, if necessary. This could have created recall bias. The retrospective estimation of time of ingestion and other circumstances in mass poisonings by methanol-contaminated spirits is approximate and probably inaccurate in some patients. Interindividual differences in body weight, chronic alcoholism, and comorbidities could have played a role in the outcome as well. Differences in the availability of treatment facilities in different hospitals (mode of dialysis, type of antidote, and so on) could have had an effect on the outcome but were outside the scope of this study.

Table 3. Treatment given in 100 hospitalized patients according to the outcome groups.*

Characteristic	Group 1 (n=49)		Group 2 (n=30)		Group 3 (n=21)	Total (n=100)	
	EtOH EMS–Positive (n=27)	EtOH EMS–Negative (n=22)	EtOH EMS–Positive (n=3)	EtOH EMS–Negative (n=27)	EtOH EMS–Negative (n=21)	EtOH EMS–Positive (n=30)	EtOH EMS–Negative (n=70)
Alkalization, No. (%)	8 (30)	12 (55)	2 (67)	25 (93)	20 (95)	10 (33)	57 (81)
Ethanol, No. (%)	21 (78)	19 (86)	2 (67)	18 (67)	16 (76)	23 (77)	53 (76)
Fomepizole, No. (%)	6 (22)	2 (9)	2 (67)	8 (30)	7 (33)	8 (27)	17 (24)
Folate substitution, No. (%)	20 (74)	19 (86)	2 (67)	22 (81)	13 (62)	22 (73)	54 (77)
CVVHD/ CVVHDF, No. (%)	10 (37)	7 (32)	1 (33)	13 (48)	15 (71)	11 (37)	35 (50)
IHD, No. (%)	8 (30)	9 (41)	1 (33)	12 (44)	5 (24)	9 (30)	26 (37)

CVVHD/CVVHDF, Continuous venovenous hemodialysis/hemodiafiltration; *IHD*, intermittent hemodialysis.
*EtOH EMS–positive: patients with out-of-hospital ethanol administration by EMS (paramedic/medical staff); EtOH EMS–negative: patients without out-of-hospital ethanol administration by EMS (paramedic/medical staff; group 1, survivors without sequelae; group 2, survivors with sequelae; group 3, died.

Table 4. Out-of-hospital administration of ethanol by paramedic or medical staff ("first aid") versus outcomes of acute methanol poisoning in 100 patients.

Characteristic	Group 1: Survived Without Sequelae (n=49)	Group 2: Survived With Sequelae (n=30)	Group 3: Died (n=21)
Out-of-hospital ethanol administered by paramedic or medical staff (n=30) (%)	27 (90.0)	3 (10.0)	0
No out-of-hospital ethanol administered by paramedic or medical staff (n=70) (%)	22 (31.4)	27 (38.6)	21 (30.0)

There was a risk of false-positive results because of inaccurate history by the patient, relatives, bystanders, or EMS providers. However, we found no evidence of false-positive cases because the history was confirmed by negative ethanol level on admission; no patients with negative history had a positive serum ethanol level on admission.

Selection bias was present because patients who died out of the hospital were not included. The consciousness of the patients on first presentation limited the study by the absence of a randomly distributed exposure: the most severely poisoned patients often did not receive out-of-hospital ethanol. There was no specific training of out-of-hospital providers and feedback to improve adherence with the protocol. An allocation bias was present in the study, caused by systematic differences other than intervention (out-of-hospital ethanol) between the groups analyzed, because the group without out-of-hospital ethanol was more severely acidotic on admission to the hospital.

DISCUSSION

Poor outcome in methanol poisoning is related to late diagnosis and delayed initiation of treatment with antidote, be it fomepizole or ethanol. In our study, both positive serum ethanol level on admission and receipt of out-of-hospital ethanol were associated with improved survival during the Czech Republic mass methanol outbreak. Our data support the use of ethanol administration to conscious patients with suspected methanol poisoning before laboratory data are available and the diagnosis is confirmed.

Table 5. Positive serum ethanol concentration on admission to the hospital versus outcomes of acute methanol poisoning in 100 patients.

Characteristic	Group 1: Survived Without Sequelae (n=49)	Group 2: Survived With Sequelae (n=30)	Group 3: Died (n=21)
Positive serum ethanol on admission (n=42)	38 (90.5)	4 (9.5)	0
Negative serum ethanol on admission (n=58)	11 (19.0)	26 (44.8)	21 (36.2)

Based on the principle "as early as possible," out-of-hospital antidote treatment during ongoing methanol outbreaks may improve patient outcomes. The decision to start the treatment cannot be based solely on the results of an assay for toxic alcohols because this is usually not readily available.[30] During the critical period before hospitalization, a poisoned patient's condition can deteriorate because of continuing accumulation of formic and lactic acids, worsening the metabolic acidosis, histotoxic hypoxia, and outcome.[31,32]

The effect of ethanol administration may be more complex than mere blocking of alcohol dehydrogenase. In animal models of cerebral, renal, liver, and cardiac ischemia, alcohol exposure is shown to reduce ischemic reperfusion injury and prevent postischemic adhesive interactions between leukocytes and endothelial cells, which can lead to organ dysfunction and death.[33-45] Ischemia caused by myelin sheath swelling and intra-axonal swelling plays a major role in compression-type injury to the optic nerve fibers, brain edema, and basal ganglia damage in methanol-poisoned patients.[14,15]

In an observational study of 11,850 patients hospitalized in an ICU,[46] positive blood alcohol concentration at hospital admission was associated with significantly decreased odds of 30-day all-cause mortality in critically ill patients. Several other studies showed a decrease of inhospital mortality in patients with positive blood alcohol concentration on hospital admission outside of the ICU, mainly in the patients with brain trauma.[47-53] An observational study of 6,733 patients hospitalized on trauma units demonstrated a decrease in inhospital mortality strongly associated with an increase in blood alcohol concentration (adjusted odds ratio=0.83 per 100 mg/dL unit change in blood alcohol concentration; 95% confidence interval 0.80 to 0.85; $P<.001$).[54]

In our study, the ethanol EMS–positive patients were less acidotic on admission to hospitals, with time to presentation and serum methanol level on admission similar to that of the ethanol EMS–negative patients. This might indicate effective blocking of the alcohol dehydrogenase enzyme in the pretreated patients. The out-of-hospital ethanol group was still able to increase ventilation adequately despite the ethanol treatment, indicating that modest administration of ethanol itself does not alter patients' ability to compensate for metabolic acidosis.

During outbreaks of mass methanol poisonings, we recommend that conscious adults with a strong suspicion of methanol poisoning receive out-of-hospital ethanol before confirmation of the diagnosis is available, with a serum ethanol goal of at least 100 mg/dL. This approach is even more important if the distance to the hospital is long or other factors may delay the definite diagnosis. Given a standard regimen, a worst-case scenario would mean that a certain number of patients will be given a limited amount of ethanol unnecessarily, which can be considered acceptable from a risk-benefit point of view.

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Author contributions: SZ and DP conceived of the study and wrote the first draft of the article. SZ, DP, and KEH collected the data. PU, ON, KK, and PD examined the patients and estimated the prevalence of visual and central nervous system damage in the population of methanol-poisoned patients. PU, ON, KK, PD, IK, ME, and KEH interpreted the data. IK provided the toxicologic measurements of methanol, ethanol, and formic acid. TN, JB, and MK conducted the statistical analysis. ME initiated the process of extracting the out-of-hospital ethanol administration from the original epidemiologic data. KEH participated in the planning of the study protocol and supervised the primary data procession. KEH and ME participated in data analysis, conceived the final format of its presentation, and critically reviewed the drafts of the article. All authors approved the final version of the article. SZ had full access to all the data in the study and had final responsibility for the decision to submit for publication. SZ takes responsibility for the paper as a whole.

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11.7. PŘÍLOHA VII

Zakharov S, **Nurieva O,** Kotikova K, Belacek J, Navratil T, Pelclova D.

Positive serum ethanol concentration on admission to hospital as the factor predictive of treatment outcome in acute methanol poisoning.

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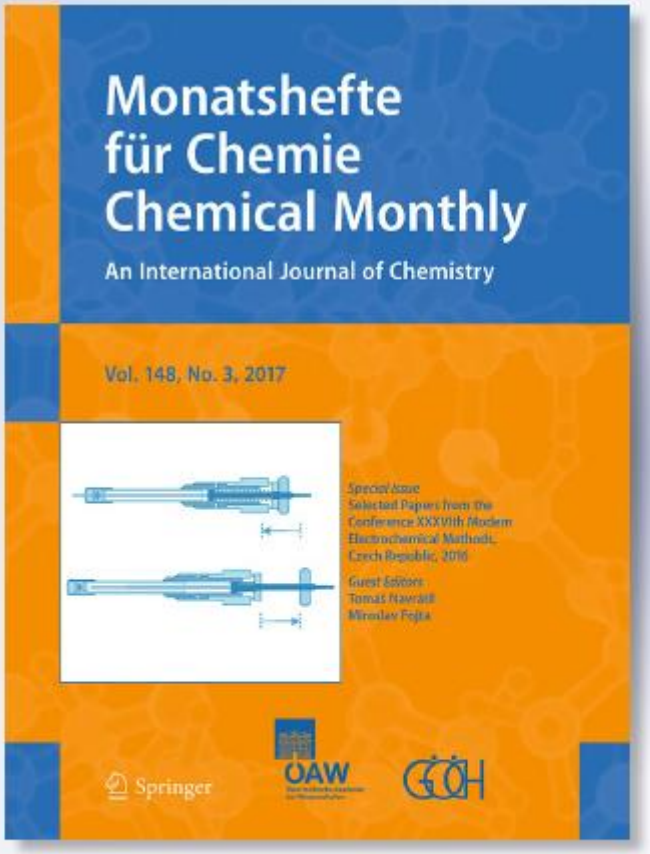

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ORIGINAL PAPER

Positive serum ethanol concentration on admission to hospital as the factor predictive of treatment outcome in acute methanol poisoning

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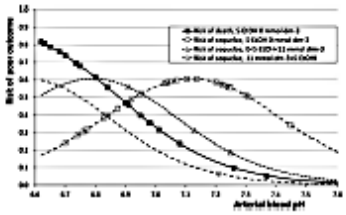
Abstract Mass methanol poisonings present a serious problem for health systems worldwide, with poor outcome associated with delayed treatment. Positive pre-hospital serum ethanol concentration may have predictive value as the prognostic factor of the treatment outcome. We studied the effect of positive serum ethanol level on admission to hospital on survival in patients treated during the Czech methanol outbreak during 2012–2014. Cross-sectional cohort study was performed in 100 hospitalized patients with confirmed methanol poisoning. Pre-hospital ethanol was administered in 42 patients (by paramedic/medical staff to 30 patients and self-administered by 12 patients before admission); 58 patients did not receive pre-hospital ethanol. Forty-two patients had detectable serum ethanol concentration on admission to hospital [median 18.3 (IQR 6.6–32.2) mmol dm^{-3}]. Pre-hospital ethanol administration by paramedic/medical staff had a significant effect on survival without visual and CNS sequelae when adjusted for arterial blood pH on admission (OR 8.73; 95 % CI 3.57–21.34; $p < 0.001$). No patients receiving pre-hospital ethanol died compared with 21 not receiving ($p < 0.001$). Positive serum ethanol concentration on admission to hospital was a predictor for survival without health sequelae when adjusted for arterial blood pH (OR 8.10; 95 % CI 2.85–23.02; $p < 0.001$). The probability of visual and CNS sequelae in survivors reduced with increasing serum ethanol concentration on admission.

Graphical abstract



Keywords Methanol poisoning · Pre-hospital ethanol administration · First aid in methanol poisoning · Factors predictive of survival · Prognosis · Sequelae of poisoning

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Introduction

Mass and cluster acute methanol poisonings due to the consumption of illicit alcohol occur frequently throughout the world [1–4]. Sporadic methanol poisonings occur either

intentionally through the abuse of methanol-containing fluids or attempted suicide or unintentionally through the misuse or occupational accident with products containing methanol as a solvent [5–7]. In the absence of protective ethanol concentration in blood serum, methanol is metabolized by hepatic alcohol dehydrogenase enzyme (cytosolic ADH1) to the highly toxic formic acid, which inhibits mitochondrial respiration [8, 9]. The accumulation of formic acid leads to the metabolic acidosis with anion gap increase, optic nerve and retinal nerve fibers damage, and necrosis of basal ganglia [10–13]. Ethanol as an antidote prevents the toxic metabolite formation by competitive blocking the ADH; therefore, its timely administration is important for successful treatment [14].

Ethanol has 10–12 times higher affinity for ADH than methanol and its serum concentration of 22–33 mmol dm^{-3} is sufficient to completely block the metabolism of methanol to formaldehyde, and on the second step to formate [15, 16]. In hospitals, the indications for ethanol administration are either a documented plasma methanol concentration of more than 6.2 mmol dm^{-3}, a high osmolal gap with documented the recent history of ingesting toxic amounts of methanol, or a metabolic acidosis with history or strong clinical suspicion of poisoning [17, 18].

To prevent high morbidity and mortality of methanol poisoning, ethanol should be administered as soon as possible after methanol ingestion [14, 19]. Its wide availability in the community makes it suitable antidote for a pre-hospital 'first aid' in the cases of suspicious toxic alcohol ingestion. We addressed this question during a recent methanol mass poisoning in the Czech Republic [20, 21]. In this study, we aimed to evaluate serum ethanol concentration on admission to hospital as the factor predictive of treatment outcome in patients with acute methanol poisoning during a methanol epidemic.

Results and discussion

During the Czech mass methanol poisoning outbreak in 2012–2014, 137 patients were poisoned and 106 of them were treated in hospitals. Of them, blood samples for serum ethanol measurement were not taken before hospital antidote treatment with intravenous ethanol, in six patients. Since serum ethanol concentration on admission before hospital treatment was the key variable for the study, these six patients were excluded from further analysis. The patients who died outside hospital ($n = 31$) were excluded from the study. Thus, 100 patients with median age 54 (interquartile range, IQR 38–61) years, 79 males and 21 females, were included in the study.

The administration of pre-hospital ethanol was identified in detailed histories taken on admission by research staff (corroborated by the laboratory analysis). Thirty patients received pre-hospital ethanol from medical or paramedical staff and twelve patients self-administered ethanol shortly before admission to hospital. The remaining 58 patients did not receive pre-hospital ethanol from any sources before presentation.

Demographic and laboratory admission data are presented in Table 1, separated according to outcome. Severity of metabolic acidosis in the patients on admission to hospital is characterized by arterial blood pH, pCO_2, HCO_{3-}, base deficit (BD), anion gap (AG), and serum lactate (Table 2). Data are presented as medians with IQR, because serum methanol, ethanol (EtOH), and osmolal gap (OG) in all groups, pH and lactate in Group I, and pCO_2 and AG in Group III were not normally distributed. Forty-two patients had detectable ethanol before hospital antidote treatment, with a median concentration of 18.3 mmol dm^{-3} (IQR 6.6–32.2 mmol dm^{-3}). The median serum ethanol on admission in the patients with pre-hospital administration by paramedics/medical staff was 18.3 mmol dm^{-3} (IQR 7.1–28.1 mmol dm^{-3}). The median serum ethanol on admission in the patients with pre-hospital self-administration was higher: 30.6 mmol dm^{-3} (6.4–81.9 mmol dm^{-3}). The serum methanol concentration on admission in EtOH-positive patients did not statistically differ from that in EtOH-negative patients; however, they were less acidotic and had lower serum glucose concentration on admission.

Clinical features on admission included visual and gastrointestinal disturbances, dyspnea, chest pain, and coma in most severely poisoned patients (Table 3). Other features included fatigue, headache, dizziness, somnolence, anxiety, alcoholic delirium, tremor, seizures, and cardiac and respiratory arrest. The median ethanol concentration was higher in patients without clinical symptoms on admission [10.9 mmol dm^{-3} (1.1–29.8 mmol dm^{-3})] than in those with clinical features [0 mmol dm^{-3} (0–5 mmol dm^{-3}); $p = 0.014$]. Detailed information about the post-admission treatment given in hospitals is presented in Table 4.

Outcome and prognosis

EtOH-positive patients had a lower rate of mortality and a higher rate of survival without visual and CNS sequelae than EtOH-negative patients (all $p < 0.001$; Table 5).

A strong positive correlation was found between the pre-hospital ethanol administration by paramedics/medical staff and serum ethanol concentration on admission ($r = 0.713$, $p < 0.001$). Furthermore, strong positive correlations were found between the serum ethanol on admission and:

Table 1 Demographic and laboratory data on admission in hospitalized patients, according to the outcome groups (medians with IQR)

		Age/ years	Time to treatment/ h	Serum methanol/ mmol dm^{-3}	Serum ethanol/ mmol dm^{-3}	Serum formate/ mmol dm^{-3}	Osmolal Gap/ mmol kg^{-1}	Serum glucose/ mmol dm^{-3}
	EtOH+ ($n = 30$)	55	25	18.6	18.3	6.9	47	6.2
		47–64	17–48	9.1–43.1	7.1–28.1	1.2–13.0	21–73	5.7–7.6
	EtOH− ($n = 70$)	52	48	29.3	0.0	14.7	45.4	8.3
		37–60	24–48	13.0–56.3	0.0–0.0	11.7–16.7	23–77	6.2–13
Group I ($n = 49$)	EtOH+ ($n = 27$)	54	26	15.6	19.3	4.9	36	6.0
		47–62	14–48	9.2–41.5	9.3–29.8	1.0–11.3	22–73	5.7–7.2
	EtOH− ($n = 22$)	52	24	21.4	1.6	13.2	26	6.6
		35–58	22–48	12.3–31.8	0.0–25.0	7.9–15.3	19–44	6.1–8.2
Group II ($n = 30$)	EtOH+ ($n = 3$)	65	24	30.9	5.0	13.5	52	7.7
		56–69	21–36	18.1–69.8	3.6–11.2	13.5–13.5	33–86	7.1–10.2
	EtOH− ($n = 27$)	48	48	50.6	0.0	15.4	64	7.6
		37–58	30–50	25.0–82.1	0.0–0.0	13.5–18.5	39–100	6.0–11.3
Group III ($n = 21$)	EtOH+ ($n = 0$)	–	–	–	–	–	–	–
	EtOH− ($n = 21$)	58	48	34.1	0.0	15.5	65	12.7
		45–63	38–52	21.6–59	0.0–0.0	12.8–16.0	45–136	10.3–16.1
Total ($n = 100$)		54	41	28.7	0.0	14.4	46.8	7.3
		38–61	24–48	12.3–54.9	0.0–12.7	8.9–16.6	21.7–75.9	6.0–11.2
$P_{(EtOH+ \text{ vs. } EtOH-)}$		0.185	0.090	0.068	<0.001***	0.005**	0.202	<0.001***
$P_{I(EtOH+ \text{ vs. } EtOH-)}$		0.212	0.636	0.459	0.883	0.093	0.151	0.220
$P_{II(EtOH+ \text{ vs. } EtOH-)}$		0.070	0.266	0.643	0.250	–	0.706	0.848
P_{I-II}		0.581	0.030*	0.004**	<0.001***	0.044*	0.013*	0.041*
P_{I-III}		0.255	0.005**	0.080	<0.001***	0.202	0.015*	<0.001***
P_{II-III}		0.138	0.351	0.204	<0.001***	0.924	0.541	0.015*

EtOH+, patients with pre-hospital ethanol administration by paramedics/medical staff; EtOH−, patients without pre-hospital ethanol administration by paramedics/medical staff; Group I, survivors without sequelae; Group II, survivors with sequelae; Group III, died, IQR, interquartile range

P_I, P_{II}, P_{III}—results of t test (two-sample assuming equal and unequal variances, respectively) of difference in laboratory parameters between the subgroups of patients with and without pre-hospital ethanol administration in Groups I, II, and III [* $\alpha \leq 0.05$; ** $\alpha \leq 0.01$; *** $\alpha \leq 0.001$ (α-significance level)]. To convert from mmol dm^{-3} to mg 0.1 dm^{-3}, use the following conversion factors: methanol—3.205; ethanol—4.608; formate—4.603; glucose—18.018

(a) survival versus death ($r = 0.418$, $p < 0.001$);
(b) survival without sequelae versus poor outcome (death or survival with sequelae; $r = 0.711$, $p < 0.001$); and
(c) survival without sequelae versus survival with sequelae versus death ($r = 0.693$, $p < 0.001$).

These correlations were strong and significant for the variable "pre-hospital ethanol administration by paramedics/medical staff" for all three variants of outcome division as well ($r = 0.338$; $r = 0.537$; and $r = 0.531$, respectively; all $p < 0.001$). In spite of the fact that there was no difference in the state-of-consciousness on the arrival of paramedics/medical staff, most of the patients with Glasgow coma scale (GCS) under 10 were not administered ethanol. Even after excluding the patients with low GCS (under 10) from the analyzed data set, the association remained significant:

(a) survival versus death ($r = 0.355$; $p = 0.001$);
(b) survival without sequelae versus poor outcome (death or survival with sequelae; $r = 0.689$, $p < 0.001$); and
(c) survival without sequelae versus survival with sequelae versus death ($r = 0.681$; $p < 0.001$).

In the univariate analysis, both serum ethanol concentration on admission and pre-hospital ethanol administration by paramedics/medical staff were significant variables for survival without sequelae (Table 6). In the bivariate regression models, the combinations of either variable, "serum ethanol on admission", and "pre-hospital ethanol administration" with the variable "arterial blood pH on admission" explained 55.4 and 48.9 % of dispersion in treatment outcomes, respectively (Table 7).

The patients with positive serum ethanol on admission had the odds ratio of survival without sequelae versus poor outcome (death or sequelae) of 8.10 (2.85–23.02 95 % CI; $p < 0.001$) when adjusted on the degree of acidemia (arterial blood pH on admission).

Table 2 Severity of metabolic acidosis on admission in hospitalized patients, according to the outcome groups (medians with IQR)

		pH	pCO_2/kPa	HCO_{3-} / mmol dm^{-3}	Base deficit/mmol dm^{-3}	Anion gap/mmol dm^{-3}	Serum lactate/mmol dm^{-3}
	EtOH+ ($n = 30$)	7.34	4.5	18.4	−6.1	20.3	2.5
		7.20–7.42	3.5–4.8	11.6–22.6	−1.5 to −14.6	18.3–28.6	1.9–3.6
	EtOH− ($n = 70$)	7.03	4.0	6.8	−23.2	32.3	6.0
		6.79–7.26	2.7–4.7	4.1–13.5	−11.3 to −29	22.3–39.8	1.9–9.3
Group I ($n = 49$)	EtOH+ ($n = 27$)	7.36	4.6	20.9	−3.6	20.0	2.5
		7.25–7.42	3.9–4.9	12.8–22.8	−1.2 to −12.8	18.1–26.8	1.9–3.4
	EtOH− ($n = 22$)	7.31	4.3	18.5	−4.5	23.2	2.1
		7.25–7.41	3.6–5.0	8.8–22.7	−1.7 to −15.6	18.2–28.5	1.7–4.0
Group II ($n = 30$)	EtOH+ ($n = 3$)	7.16	2.6	5.9	−22.1	30.9	4.8
		7.01–7.18	2.3–3.3	4.7–8.7	−19.6 to −26.1	29.8–31.9	3.2–6.3
	EtOH− ($n = 27$)	7.02	2.9	5.1	−25.4	32.7	3.2
		6.83–7.17	1.9–3.6	3.6–9.3	−19.1 to −27.5	25.3–37.7	1.4–7.4
Group III ($n = 21$)	EtOH+ ($n = 0$)	–	–	–	–	–	–
	EtOH− ($n = 21$)	6.79	4.5	5.2	−29	40.4	9.4
		6.65–6.93	3.5–6.1	3.9–7.7	−26.9 to −31.9	34.8–45.1	6.7–12.9
Total ($n = 100$)		7.18	4.1	8.8	−17.8	28.3	3.6
		6.89–7.34	2.8–4.8	4.7–19.5	−3.7 to −27.7	19.4–36.3	1.9–7.8
$P_{(EtOH+ \text{ vs. } EtOH-)}$		<0.001***	0.587	<0.001***	<0.001***	<0.001***	<0.001***
$P_{I(EtOH+ \text{ vs. } EtOH-)}$		0.373	0.759	0.300	0.905	0.418	0.449
$P_{II(EtOH+ \text{ vs. } EtOH-)}$		0.601	0.976	0.939	0.961	0.666	0.991
$P_{I–II}$		<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	0.111
$P_{I–III}$		<0.001***	0.181	<0.001***	<0.001***	<0.001***	<0.001***
$P_{II–III}$		<0.001***	0.041*	0.401	0.012*	0.042*	<0.001***

EtOH+, patients with pre-hospital ethanol administration by paramedics/medical staff; EtOH−, patients without pre-hospital ethanol administration by paramedics/medical staff; pH, arterial blood pH on admission; HCO_{3-}, arterial blood bicarbonate on admission; Group I, survivors without sequelae; Group II survivors with sequelae; Group III, died; IQR, interquartile range

P_I, P_{II}, P_{III}—results of t test (two-sample assuming equal and unequal variances, respectively) of difference in laboratory parameters between the subgroups of patients with and without pre-hospital ethanol administration in Groups I, II, and III [* $\alpha \leq 0.05$; ** $\alpha \leq 0.01$; *** $\alpha \leq 0.001$ (α-significance level)]. To convert serum lactate from mmol dm^{-3} to mg 0.1 dm^{-3}, use the conversion factor 9.009. To convert bicarbonate and base deficit from mmol dm^{-3} to mEq dm^{-3}, use the conversion factor 1.0. To convert kPa to mmHg (torr), use the conversion factor 7.501

Furthermore, the patients with pre-hospital ethanol administration by paramedics/medical staff had the odds ratio of survival without sequelae versus poor outcome (death or sequelae) of 8.73 (3.57–21.34 95 % CI; $p < 0.001$) when adjusted on arterial blood pH on admission. Inclusion of any other independent variable in the logistic regression model did not lead to further increase of its ability to explain the dispersion in treatment outcomes.

The univariate and bivariate regression models for three variants of treatment outcome analyzed separately (survival without sequelae versus survival with sequelae versus death) are presented on Fig. 1. Serum ethanol concentration on admission, severity of metabolic acidosis, and pre-hospital ethanol administration ("first aid") are the most significant variables for the outcome of treatment.

The logistic regression of probability of death or survival with sequelae versus concentration of serum ethanol and arterial blood pH on admission is shown in Fig. 2. The probability of death decreased exponentially with the increase of arterial blood pH. All who died had negative serum ethanol on admission; however, the probability of developing sequelae among the survivors was dependent not only on the degree of acidemia, but also on the serum ethanol concentration on admission, with a significant leftward shift of the peak of the curve. This implies that an increasing ethanol concentration was protective against visual and CNS damage given the same arterial blood pH. For example, the patients with arterial blood pH 7.0: the probability of developing sequelae was 59 % (negative serum ethanol) versus 41 % (serum ethanol <11 mmol dm^{-3}) versus 16 % (serum ethanol >11 mmol dm^{-3}).

Table 3 Clinical symptoms on admission in hospitalized patients according to the outcome groups

		No symptoms, *n* (%)	VD, *n* (%)	GI, *n* (%)	D, *n* (%)	CP, *n* (%)	RA, *n* (%)	C, *n* (%)
Group I ($n = 49$)	EtOH+ ($n = 27$)	16 (59 %)	4 (15 %)	7 (26 %)	1 (4 %)	1 (4 %)	0 (0 %)	0 (0 %)
	EtOH− ($n = 22$)	6 (27 %)	10 (45 %)	18 (82 %)	10 (45 %)	1 (5 %)	0 (0 %)	4 (18 %)
Group II ($n = 30$)	EtOH+ ($n = 3$)	1 (33 %)	3 (100 %)	2 (67 %)	1 (33 %)	0 (0 %)	0 (0 %)	1 (33 %)
	EtOH− ($n = 27$)	0 (0 %)	8 (30 %)	8 (30 %)	8 (30 %)	1 (4 %)	0 (0 %)	8 (30 %)
Group III ($n = 21$)	EtOH− ($n = 21$)	0 (0 %)	12 (57 %)	10 (48 %)	11 (53 %)	7 (33 %)	3 (14 %)	15 (71 %)
Total ($n = 100$)	EtOH+ ($n = 30$)	17 (57 %)	7 (23 %)	9 (30 %)	2 (7 %)	1 (3 %)	0 (0 %)	1 (3 %)
	EtOH− ($n = 70$)	6 (9 %)	30 (43 %)	36 (51 %)	29 (41 %)	9 (13 %)	3 (4 %)	27 (39 %)
$P_{tot(EtOH+ vs. EtOH-)}$	*p*	<0.001***	0.064	0.048	0.001***	0.146	0.250	<0.001***
	OR (CI)	13.95 (4.6–42.1)	0.41 (0.15–1.07)	0.41 (0.16–1.01)	0.10 (0.02–0.46)	0.23 (0.03–1.93)	0.000 (–)	0.06 (0.01–0.43)
$P_{I(EtOH+ vs. EtOH-)}$	*p*	0.025	0.018*	<0.001***	<0.001***	0.88	1.000	0.021
	OR (CI)	3.88 (1.15–13.04)	0.21 (0.05–0.81)	0.08 0.02–0.31)	0.05 (0.01–0.40)	0.81 (0.05–13.70)	0.000 (–)	0.000 (–)
$P_{II(EtOH+ vs. EtOH-)}$	*p*	0.002	0.016*	0.197	0.894	0.735	1.000	0.894
	OR (CI)	0.000 (–)	0.000 (–)	4.75 (0.38–60.15)	1.19 (0.09–15.04)	0.000 (–)	0.000 (–)	1.19 (0.09–15.04)

EtOH+, patients with pre-hospital ethanol administration by paramedics /medical staff; EtOH−, patients without pre-hospital ethanol administration by paramedics/medical staff; Group I, survivors without sequelae; Group II, survivors with sequelae; Group III, died, VD, visual disturbances; GI, gastrointestinal symptoms, D, dyspnea, CP, chest pain, C, coma, RA, respiratory arrest

Chi^2-test [* $\alpha \leq 0.05$; ** $\alpha \leq 0.01$; *** $\alpha \leq 0.001$ (α-significance level)]

Table 4 Treatment given in hospitalized patients according to the outcome groups

		Alkalization	Ethanol	Fomepizole	Folates	CVVHD/CVVHDF	IHD
Group I ($n = 49$)	EtOH+ ($n = 27$)	8 (30 %)	21 (78 %)	6 (22 %)	20 (74 %)	10 (37 %)	8 (30 %)
	EtOH− ($n = 22$)	12 (55 %)	19 (86 %)	2 (9 %)	19 (86 %)	7 (32 %)	9 (41 %)
Group II ($n = 30$)	EtOH+ ($n = 3$)	2 (67 %)	2 (67 %)	2 (67 %)	2 (67 %)	1 (33 %)	1 (33 %)
	EtOH− ($n = 27$)	25 (93 %)	18 (67 %)	8 (30 %)	22 (81 %)	13 (48 %)	12 (44 %)
Group III ($n = 21$)		20 (95 %)	16 (76 %)	7 (33 %)	13 (62 %)	15 (71 %)	5 (24 %)
Total ($n = 100$)	EtOH+ ($n = 30$)	10 (33 %)	23 (77 %)	8 (27 %)	22 (73 %)	11 (37 %)	9 (30 %)
	EtOH− ($n = 70$)	57 (81 %)	53 (76 %)	17 (24 %)	54 (77 %)	35 (50 %)	26 (37 %)
$P_{tot (EtOH+ vs. EtOH-)}$	*p*	<0.001***	0.919	0.801	0.683	0.220	0.493
	OR (CI)	0.11 (0.04–0.30)	1.05 (0.39–2.89)	1.13 (0.43–3.01)	0.82 (0.31–2.18)	0.58 (0.24–1.39)	0.73 (0.29–1.82)
$P_{I (EtOH+ vs. EtOH-)}$	*p*	0.078	0.440	0.216	0.288	0.703	0.409
	OR (CI)	0.35 (0.11–1.14)	0.55 (0.12–2.52)	2.86 (0.5–15.85)	0.45 (0.10–2.00)	1.26 (0.38–4.14)	0.61 (0.19–1.99)
$P_{II (EtOH+ vs. EtOH-)}$	*p*	0.156	1.000	0.197	0.543	0.626	0.713
	OR (CI)	0.16 (0.01–2.63)	1.00 (0.08–12.56)	4.75 (0.4–60.15)	0.46 (0.03–6.06)	0.54 (0.04–6.67)	0.63 (0.05–7.75)

EtOH+, patients with pre-hospital ethanol administration by paramedics/medical staff; EtOH−, patients without pre-hospital ethanol administration by paramedics/medical staff; Group I, survivors without sequelae; Group II, survivors with sequelae; Group III, died; CVVHD/HDF, continuous veno-venous hemodialysis/hemodiafiltration; IHD, intermittent hemodialysis

Chi^2 test [* $\alpha \leq 0.05$; ** $\alpha \leq 0.01$; *** $\alpha \leq 0.001$ (α-significance level)]

Table 5 Pre-hospital administration of ethanol versus outcomes of acute methanol poisonings ($n = 100$)

	Group I: survived without sequelae	Group II: survived with sequelae	Group III: died
Pre-hospital ethanol administered by paramedics/medical staff ($n = 30$)	27 (90.0 %)	3 (10.0 %)	0 (0.0 %)
Pre-hospital ethanol not given by paramedics/medical staff ($n = 70$)	22 (31.4 %)	27 (38.6 %)	21 (30.0 %)
p	<0.001***	0.004**	<0.001***
OR (CI)	19.64 (5.38–71.7)	0.2 (0.05–0.64)	0.0 (–)
Pre-hospital ethanol, including self-administration ($n = 42$)	38 (90.5 %)	4 (9.5 %)	0 (0.0 %)
Pre-hospital ethanol not given ($n = 58$)	11 (19.0 %)	26 (44.8 %)	21 (36.2 %)
p	<0.001***	<0.001***	<0.001***
OR (CI)	40.6 (12.0–137.7)	0.1 (0.04–0.41)	0.0 (–)

Chi^2-test [* $\alpha \leq 0.05$; ** $\alpha \leq 0.01$; *** $\alpha \leq 0.001$ (α-significance level)]

Table 6 Parameters of the univariate analysis significant for survival without sequelae

	Intercept	β	SE	OR	LE 95 % CI	UE 95 % CI	p	Cox and Snell R^2	Nagelkerke R^2	Hosmer–Lemeshow R^2
S-EtOH	–6.319	2.576	0.504	**13.139**	4.892	35.291	0.000	0.439	0.586	0.417
pH	–4.013	2.253	0.411	9.515	4.249	21.308	0.000	0.405	0.540	0.375
HCO_{3-}	–2.839	1.527	0.284	4.602	2.637	8.033	0.000	0.321	0.427	0.279
"First aid"	–2.197	2.977	0.661	**19.636**	5.378	71.703	0.000	0.273	0.365	0.230
GCS	–2.086	1.556	0.441	4.742	1.997	11.258	0.000	0.141	0.189	0.110
S-MetOH	–1.841	0.941	0.279	2.563	1.485	4.425	0.001	0.122	0.162	0.094
Time	–1.917	1.068	0.352	2.910	1.459	5.804	0.002	0.115	0.153	0.088
S-Lactate	–1.396	0.933	0.332	2.541	1.325	4.873	0.005	0.105	0.142	0.082

OR, odds ratio; LE 95 % CI, lower endpoint of 95 % confidence interval; UE 95 % CI, upper endpoint of 95 % confidence interval; "First aid", pre-hospital ethanol administration by paramedics/medical staff; pH, arterial blood pH on admission;HCO_{3-}, arterial blood bicarbonate on admission; GCS, Glasgow coma scale; S-EtOH, serum ethanol on admission; S-MetOH, serum methanol on admission; S-Lactate, serum lactate on admission; Time, time span between methanol ingestion and the treatment

Bold value indicates statistically significant p values ($p < 0.05$)

The logistic regression of probability of poor outcome (death or sequelae) in the patients with pre-hospital ethanol administration by the paramedics/medical staff versus probability in the patients without pre-hospital ethanol administration is shown in Fig. 3.

Serum ethanol on admission and prognostic parameters of treatment outcome

The poor outcome in methanol poisonings is primarily associated with the late diagnosis and delayed initiation of treatment with antidote, be it fomepizole or ethanol [22–24]. Severity of metabolic acidosis on admission is known prognostic parameter of poor outcome (death or long-term visual and/or central nervous system sequelae) in acute methanol poisoning [25–28]. Timely correction of acidemia and the elimination of formic acid by hemodialysis are one of the crucial issues for successful treatment [29–33].

In our study, the EtOH-positive patients were less acidotic on admission to hospitals with significantly higher arterial blood pH and bicarbonate, and lower lactate, base deficit, and anion gap, with no difference in time to presentation or serum methanol on admission as compared with the EtOH-negative patients. This indicates an effective blocking of the ADH enzyme in the pre-treated patients. The pre-hospital ethanol group was still able to hyperventilate adequately in spite of the ethanol treatment indicating that a modest administration of ethanol itself does not alter the patients' ability to hyperventilate, and thus not removing this important compensatory mechanism [34, 35].

There were significantly more asymptomatic patients on admission to hospitals in the EtOH-positive group. In Group I (survivors without sequelae), there were also fewer patients

Table 7 Bivariate logistic regression for pre-hospital ethanol/serum ethanol on admission and the parameters significant for survival without sequelae

		Intercept	β_1	SE_1	p_1	β_2	SE_2	p_2	Adjusted OR (exp β_1)	LE 95 % CI	UE 95 % CI	Hosmer–Lemeshow R^2
pH	First aid	−5.894	2.166	0.456	0.000	2.725	0.809	0.001	**8.726**	**3.567**	**21.344**	0.489
HCO_3_	First aid	−4.090	1.258	0.304	0.000	2.308	0.720	0.001	3.519	1.941	6.380	0.373
GCS	First aid	−4.101	2.868	0.685	0.000	1.458	0.523	0.005	17.595	4.593	67.404	0.299
S-MetOH	First aid	−4.139	2.974	0.698	0.000	0.972	0.333	0.004	19.562	4.978	76.877	0.296
S-Lactate	First aid	−3.914	3.434	0.824	0.000	0.913	0.411	0.027	30.989	6.158	155.943	0.349
Time	First aid	−4.027	2.857	0.709	0.000	1.075	0.412	0.009	17.415	4.342	69.843	0.288
pH	S-EtOH	−8.393	2.092	0.533	0.000	1.817	0.478	0.000	**8.103**	**2.852**	**23.018**	0.554
HCO_3_	S-EtOH	−7.188	2.137	0.518	0.000	1.018	0.339	0.003	8.476	3.070	23.404	0.485
GCS	S-EtOH	−7.824	2.417	0.490	0.000	1.435	0.641	0.025	11.216	4.289	29.328	0.462
S-MetOH	S-EtOH	−8.130	2.554	0.534	0.000	0.933	0.378	0.014	12.863	4.514	36.660	0.461

OR, odds ratio; LE 95 % CI, lower endpoint of 95 % confidence interval; UE 95 % CI, upper endpoint of 95 % confidence interval; "First aid", pre-hospital ethanol administration by paramedics/medical staff; "S-EtOH" - serum ethanol on admission; pH, arterial blood pH on admission; HCO_3_, arterial blood bicarbonate on admission; GCS, Glasgow coma scale; S-MetOH, serum methanol on admission; S-EtOH, serum ethanol on admission; S-Lactate, serum lactate on admission; Time, time span between methanol ingestion and the treatment

Bold value indicates statistically significant p values ($p < 0.05$)

with dyspnea and symptoms of visual toxicity at presentation among the EtOH-positive patients comparing the EtOH-negative patients. Only one patient administered pre-hospital ethanol with GCS 10 fell into a coma on admission to the hospital; this patient had severe acidemia on admission to hospital with high serum lactate and low methanol, suggesting that most of it had already been metabolized to toxic formic acid. In other patients who received pre-hospital ethanol administration, no deterioration of the state-of-consciousness was registered after admission.

The significant association was found between the outcome of treatment and both serum ethanol concentration on admission and pre-hospital ethanol administration by paramedics/medical staff in our study. The positive association remained strong after the elimination of patients with GCS $\leq$10 on the first presentation. Logistic regression analysis demonstrated that serum ethanol concentration on admission was significant variable for the treatment outcome when adjusted for the degree of acidemia and the state-of-consciousness on admission.

The probability of poor outcome (death or sequelae) decreased exponentially with increasing arterial blood pH, but the rate of decrease was higher in the patients with pre-hospital ethanol administration. Finally, the probability of survival with visual and/or CNS sequelae depended on the serum ethanol concentration.

In summary, the present data document the significant association between positive serum ethanol concentration on admission to hospital and better treatment outcome in acute methanol poisoning. This fact supports the recommendation on the potential benefit of the pre-hospital administration of ethanol on outcome during an on-going outbreak of methanol poisoning [21]: given a standard regimen, a worst-case scenario would mean that a certain number of patients will be given a limited amount of ethanol unnecessarily, which can be considered acceptable from a risk–benefit point of view.

Strength and limitations

The limitations of this study can be attributed to certain confounders, as it was not a randomized controlled trial, leaving the possibility of inherent bias during the comparisons. The numbers of the patients in both groups were relatively small (even if by far the largest of its kind), and most of the patients in both groups were the so-called "late-presenters". Despite the limitations and confounders, the study provides important data on the effect of positive serum ethanol concentration on admission to hospital on the outcome of treatment during a large methanol outbreak. The essential clinical and laboratory data on admission were collected during admission to hospital using standardized forms. The groups of patients were also comparable by age, circumstances of poisoning, latency period, and size.

Conclusion

In our study, positive serum ethanol concentration on admission to hospital was associated with survival and better treatment outcome of poisoned patients during the

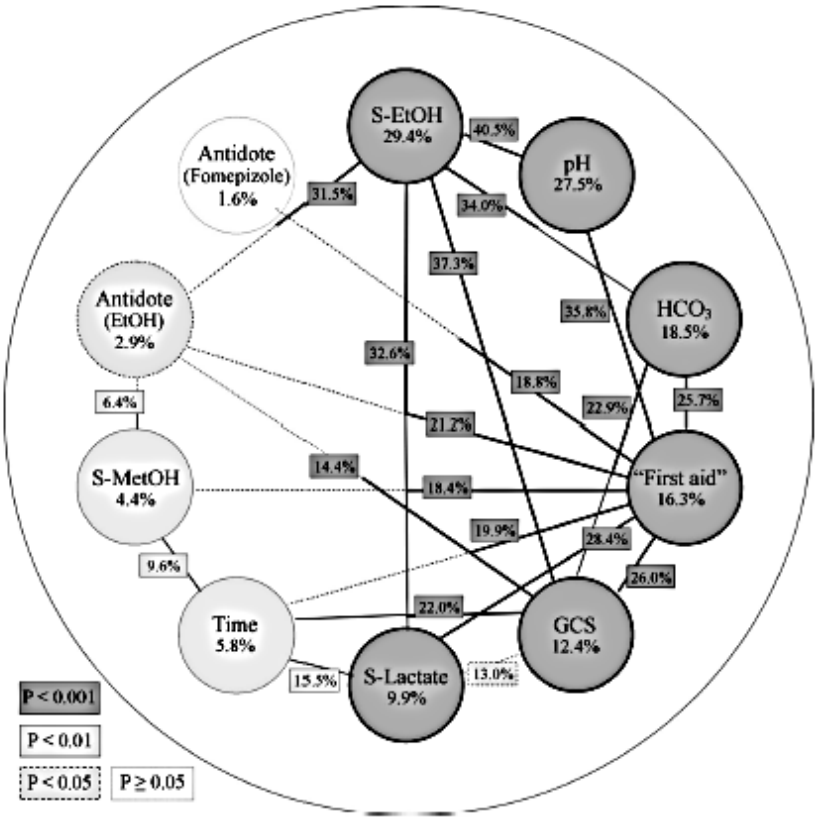

Fig. 1 Percents of explained dispersion in univariate and bivariate ordinal multinomial models for three categories of treatment outcomes (survival without sequelae versus survival with sequelae versus death). For the univariate models, see parameters and per cents inside the circles; for bivariate models, see per cents on the lines connecting parameters. S-EtOH, serum ethanol concentration on admission; pH, arterial blood pH on admission; HCO_3–, arterial blood bicarbonate on admission; "First aid", pre-hospital ethanol administration by paramedics/medical staff; GCS, Glasgow coma scale; S-Lactate, serum lactate on admission; Time, time span between methanol ingestion and the treatment; S-MetOH, serum methanol on admission; Antidote (EtOH), hospital administration of ethanol; Antidote (Fomepizole), hospital administration of fomepizole

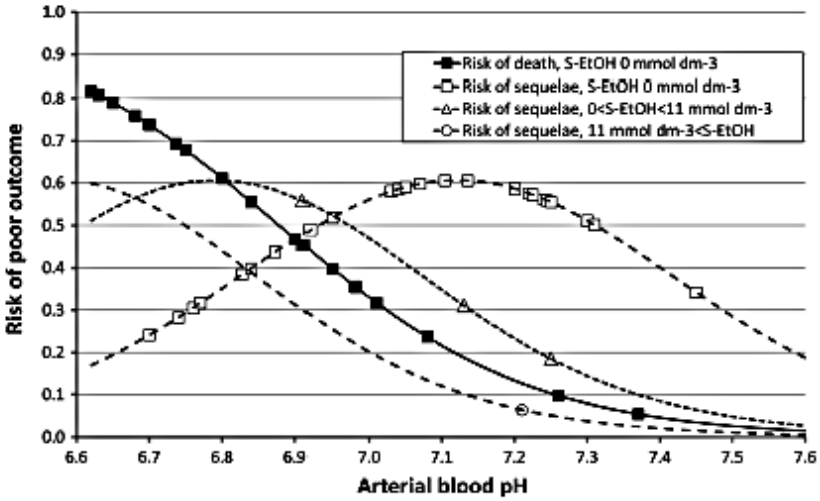

Fig. 2 Risk of death and survival with sequelae versus arterial blood pH and serum ethanol on admission. Total $n = 100$: died ($n = 21$) + survivors with sequelae ($n = 30$) + survivors without sequelae ($n = 49$). *S-EtOH* serum ethanol concentration on admission

Czech mass methanol outbreak. Our data, therefore, strongly support a recommendation that ethanol can be administered pre-hospital by paramedics/medical staff to conscious patients suspected to be poisoned with methanol before laboratory data are available and diagnosis confirmed.

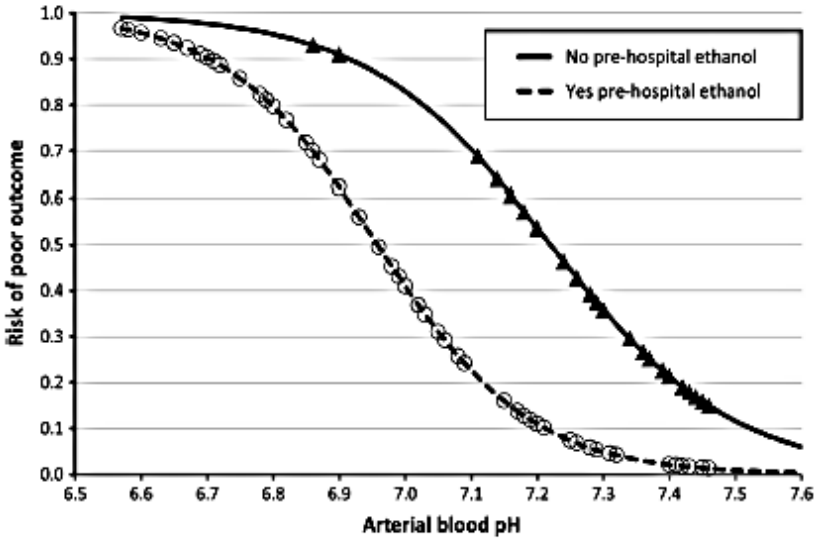

Fig. 3 Risk of poor outcome (death or sequelae) versus pre-hospital ethanol administration by paramedics/medical staff. Total $n = 100$: poor outcome [Group III (died); $n = 21$ + Group II (survivors with sequelae); $n = 30$]. Favorable outcome [Group I (survivors without sequelae); $n = 49$]

Experimental

Patients

Among 137 cases of methanol poisoning in the Czech Republic from the 3 September 2012 until the 31 August 2014, 106 patients were treated in hospitals. The discharge reports of all hospitalized patients with a confirmed diagnosis and the results of neurological and ophthalmological examinations on admission, during hospitalization, and on discharge were collected and analyzed in the TIC. A detailed history of the poisoning, and of the onset and dynamics of ocular and systemic toxicity, was obtained in a prospective manner directly from the patients or from relatives of critically ill patients upon admission to the secondary hospital.

Laboratory analyses were performed on admission. Diagnosis was established when (1) a history of recent ingestion of illicit spirits was available and serum methanol was higher than 6.2 mmol dm^{-3} and/or an osmolal gap (OG) $\geq$20 mOsm (kg $H_2O)^{-1}$ was found, or (2) there was a history/clinical suspicion of methanol poisoning, and serum methanol was above the limit of detection with at least two of the following: pH < 7.3, serum bicarbonate <20 mmol dm^{-3}, and anion gap (AG) $\geq$20 mmol dm^{-3} [36, 37].

The clinical examination protocol included complete ocular examination with the standard ophthalmologic tests (visual acuity, color vision, contrast sensitivity, perimeter, and fundus), cerebral computed tomography (CT) or magnetic resonance imaging (MRI) of the brain, and standard neurological examination. The patients were considered to have visual sequelae of acute methanol poisoning if the symptoms of toxic neuropathy of the optic nerve were documented on admission/during hospitalization, with pathologic findings on visual acuity, visual fields, color vision, contrast sensitivity, and persisting lesions on fundoscopy with other symptoms of visual damage being found on discharge from the hospitals. The patients were considered as having CNS sequelae of poisoning if symmetrical necrosis and hemorrhages of basal ganglia were present on CT or MRI of the brain.

The hospitalized patients were retrospectively divided into three groups according to their outcome: Group I: Patients who survived without sequelae; Group II: patients who survived with visual and/or CNS sequelae; and Group III: patients who died. These groups were then further divided into two subgroups 'with pre-hospital ethanol administration by paramedics/medical staff (EtOH-positive)' and 'without pre-hospital ethanol administration by paramedics/medical staff (EtOH-negative)'. Within the latter subgroup, the data from patients who self-administered ethanol shortly before presentation to hospitals were analyzed separately.

Treatment

All patients were treated in accordance to the American Association of Clinical Toxicology and the European Association of Poison Centres and Clinical Toxicologists (AACT/EAPCCT) practice guidelines on the treatment of methanol poisoning [14]. Bicarbonate 8.4 or 4.2 % solution was given intravenously as a buffer to the patients with metabolic acidosis. Fomepizole or ethanol were administered as antidotes to block ADH enzyme. Folates were administered to substitute the endogenous pool.

Enhanced elimination was performed if the patients met any of the following criteria: serum methanol higher than 15.6 mmol dm^{-3}, metabolic acidosis with arterial blood pH < 7.30, or had the signs of visual toxicity. The choice of modality of enhanced elimination was based on several factors, such as the hemodynamic stability of a patient on admission, or the severity of poisoning, and availability of dialysis equipment.

Laboratory investigations

Methanol was measured by a gas chromatographic method with flame ionization detection and a direct injection with an internal standard, limit of detection 1.9 mmol dm^{-3}, and day-to-day coefficient of variation 2.5–5.4 %. Formate was measured enzymatically using formate dehydrogenase and nicotinamide adenine dinucleotide, according to a previously published method [38, 39]. Day-to-day coefficient of variation was 5.6 %, and the upper reference limit was 0.44 mmol dm^{-3}. Serum ethanol was analyzed by gas chromatography with flame ionization detection and direct injection with an internal standard. The limit of detection was 0.87 mmol dm^{-3}, and the day-to-day coefficient of variation was 3.8–7.1 %. Osmolality was measured by the freezing point depression method on a Fiske one-ten osmometer. The reference range for the osmolal gap was −9 to 19 mOsm (kg H_2O)$^{-1}$ [40]. The osmolal contribution from ethanol was subtracted from the measured osmolality.

Statistical analyses

The laboratory and clinical data were compared using two-sample assuming unequal variances, two-sample F test for variances, bias test, and two-sample Kolmogorov–Smirnov test. The data were expressed as medians with interquartile ranges (IQR). Spearman's rank correlation, exploratory factor analysis, and Chi-square tests were used to analyze the association between different variables and the outcomes of treatment. Statistically significant parameters were subsequently used in the regression models of ordinal multinomic logistic regression based on likelihood ratio estimation. Probabilistic analysis of predictive ability of significant parameters for the poor outcome of treatment was applied using Hosmer–Lemeshow likelihood ratio R^2. All statistical calculations were carried out on the level of significance $\alpha = 0.05$.

Ethics

The study was approved by the General University Hospital Ethics Committee in Prague, Czech Republic.

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