VILNIAUS UNIVERSITETO
MEDICINOS FAKULTETAS
TARYBA

NUTARIMAS
DĖL REIKŠMINGIAUSIŲ VILNIAUS UNIVERSITETO MEDICINOS FAKULTETO 2023 METŲ MOKSLO PASIEKIMŲ TEIKIMO VILNIAUS UNIVERSITETO MOKSLININKŲ SKATINIMO UŽ REIKŠMINGUS MOKSLO PASIEKIMUS ATRANKOS KONKURSUI

Vadovaudamasi Vilniaus universiteto mokslininkų skatinimo už reikšmingus mokslo pasiekimus tvarkos aprašu, patvirtintu 2014 m. sausio 9 d. rektoriaus įsakymu Nr. R-12, Medicinos fakulteto taryba

n u t a r i a:

teikti apdovanojimui už reikšmingus mokslo pasiekimus (**nominacijų rūšis – geriausia Vilniaus universiteto mokslininkų publikacija**) dvi publikacijas:

1. **Strumila, Robertas; Lengvenytė, Aistė; Zdanavičius, Linas; Badaras, Robertas; Dlugauskas, Edgaras; Lesinskienė, Sigita;** Matiekus, Eimantas; Marcinkevičius, Martynas**;** Vencevičienė, Lina; **Utkus, Algirdas; Kaminskas, Andrius; Petrėnas, Tomas; Songailienė, Jurgita; Ambrozaitytė, Laima**. Significantly elevated phosphatidylethanol levels in recent suicide attempters, but not in depressed controls and healthy volunteers: Possible role of CRP and inflammation // Journal of psychiatric research. Oxford : Pergamon-Elsevier science Ltd. ISSN 0022-3956. eISSN 1879-1379. 2023, vol. 158, p. 245-254. DOI: 10.1016/j.jpsychires.2022.12.043. [DB: Science Citation Index Expanded (Web of Science), Scopus, Social Sciences Citation Index (Web of Science)]
2. William Morello, M.D., Ph.D., Esra Baskin, M.D., **Augustina Jankauskiene, M.D.,** Fatos Yalcinkaya, M.D., Aleksandra Zurowska, M.D., Giuseppe Puccio, M.D., Jessica Serafinelli, M.D., Angela La Manna, M.D., Grażyna Krzemień, M.D., Marco Pennesi, M.D., Claudio La Scola, M.D., Ph.D., Francesca Becherucci, M.D., et al., for the PREDICT Study Group. Antibiotic Prophylaxis in Infants with Grade III, IV, or V Vesicoureteral Reflux. N Engl J Med. 2023 Sep 14;389(11):987-997. doi: 10.1056/NEJMoa2300161. Epub 2023 Sep 12.PMID: 37702442 Clinical Trial.

Journal of Psychiatric Research 158 (2023) 245–254

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/jpsychires

Significantly elevated phosphatidylethanol levels in recent suicide attempters, but not in depressed controls and healthy volunteers

Robertas Strumila [a,b,d,*], Aiste Lengvenyte [a,b,d], Linas Zdanavicius [c], Robertas Badaras [c], Edgaras Dlugauskas [d], Sigita Lesinskiene [d], Eimantas Matiekus [e], Martynas Marcinkevicius [e], Lina Venceviciene [f], Algirdas Utkus [g], Andrius Kaminskas [g], Tomas Petrenas [g], Jurgita Songailiene [g], Laima Ambrozaityte [g]

[a] Department of Urgent and Post Urgent Psychiatry, CHU Montpellier, Montpellier, France
[b] Institute of Functional Genomics, CNRS, INSERM, University of Montpellier, Montpellier, France
[c] Centre for Toxicology, Clinic of Anaesthesiology, Reanimatology and Critical Care Medicine, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania
[d] Clinic of Psychiatry, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania
[e] Vilnius City Mental Health Center, Vilnius, Lithuania
[f] Centre for Family Medicine, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania
[g] Department of Human and Medical Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius, Lithuania

ARTICLE INFO

Keywords:
Suicide
Phosphatidylethanol
Alcohol use disorder
Suicide attempt
Depression

ABSTRACT

Introduction: Suicide is a complex transdiagnostic phenomenon. It is strongly associated with, but not exclusive to major depressive disorder (MDD). Hazardous alcohol drinking has also been linked to an increased risk of suicidal behaviours, however, it is often underreported. The study aimed to evaluate whether an objective measure of chronic alcohol use, phosphatidylethanol (PEth) could be useful as a biomarker in clinical practice.
Method: ology. The present case-control multi-centric study recruited 156 participants into three study groups: 52 patients treated for major depressive disorder (MDD), 51 individuals immediately following a suicide attempt (SA), and 53 volunteers. Sociodemographic data, medical history, and laboratory data, including PEth concentrations and C-reactive protein levels, were collected from study participants.
Results: PEth concentrations were the highest in suicide attempters (232,54 ± 394,01 ng/ml), followed by patients with MDD (58,39 ± 135,82 ng/ml), and the control group (24,45 ± 70,83 ng/ml) (Kruskall Wallis χ^2 = 12.23, df = 2, p = .002). In a multinomial logistic regression model with adjustments, PEth concentration was able to predict belonging to suicide attempters' group, but not to depression group (p = .01). Suicide attempters were also more likely to underreport their recent alcohol consumption.
Limitations: We did not analyze SA methods, psychiatric comorbidity and several other factors that might be associated with PEth levels, such as body mass index, race, and haemoglobin levels. Sample recruited in hospital settings may not be representative of the whole population. The results of this adult-only study cannot be generalized to adolescents.
Conclusions: PEth levels in recent suicide attempters significantly exceeded those of patients with MDD and controls. Suicide attempters also were more likely to underreport their alcohol consumption when questioned about their consuption. PEth might be an interesting biomarker to evaluate individuals at risk of SA.

1. Introduction

Suicide is a complex phenomenon associated with a dysregulation of multiple body regulatory systems and such maladaptive cognitive patterns as impaired decision making and impulsivity (Baldessarini et al., 2017; Lengvenyte et al., 2021a; Solano et al., 2016). It remains a major contributor to premature mortality worldwide (WHO, 2018), and some countries, such as United States (US), have witnessed increased suicide mortality in recent years (Woolf and Schoomaker, 2019). History of suicide attempt (SA) is the best-described risk factor for eventual death

* Corresponding author. Clinic of Psychiatry, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania
E-mail address: robertas.strumila@gmail.com (R. Strumila).

https://doi.org/10.1016/j.jpsychires.2022.12.043
Received 25 April 2022; Received in revised form 21 November 2022; Accepted 22 December 2022
Available online 27 December 2022

by suicide (Bostwick et al., 2016; García de la Garza et al., 2021). However, despite substantial research efforts and a proliferation of questionnaires conceived to capture suicide risk, current models fail to prove substantial clinical value (Kessler et al., 2020). Such established suicide risk factors as mental illness or childhood adversity lack specificity and the majority of individuals presenting these traits or states will never engage in suicidal behaviours (SB) (Franklin et al., 2017). Current specific treatment interventions also lack efficacy in suicidal patients (Lengvenyte et al., 2021b), further underlining the need to further explore other factors associated with SB that could be targeted in individuals at risk.

In the major conceptual models of suicide, distal risk factors represent both genetic and environmental exposures that entail an increased vulnerability to engage in SB in face of proximal risk factors, such as stressful life events or mental illness (John Mann and Rizk, 2020). One major comorbidity that might serve as an important proximal factor, but often remains understudied and unaddressed, is hazardous substance use. Studies show that substance use disorders are seldom treated in the general population (Hasin and Grant, 2015). Yet, increased mortality due to alcohol abuse and drug overdose accompanied increasing rates of suicide in the US (Woolf and Schoomaker, 2019). The US has also seen a substantial increase in alcohol use and binge drinking over the past 10–15 years (Grucza et al., 2018). Substance use disorders present a major risk factor for suicidal behaviours, with 2-to-14-fold increased risk suicidal ideation (SI), SB, and suicide (Hesse et al., 2020; Poorolajal et al., 2016).

While misuse of almost any psychoactive substance has been associated with SB (Ferrari et al., 2014), excessive alcohol use is of particular interest due to its widespread use and cultural acceptance. A recent post-mortem study in Australia has reported that higher alcohol blood concentration was associated with an increased risk for death cause to be suicide (Chitty et al., 2021). Alcohol dependence has been associated with a 2-fold increased risk to die by suicide following the initial suicidal crisis, as compared to the low-risk drinkers (Robins et al., 2021). Alcohol intoxication has also been associated with suicide by violent means (Choi et al., 2018). Alcohol use is associated with a higher risk of SA in three months following the initial evaluation in individuals with baseline SI (Richards et al., 2020). 6–8% of individuals who use alcohol have a history of SA (Cherpitel et al., 2004). However, multiple studies have shown that alcohol consumption is both frequently unexplored by healthcare professionals and misreported by individuals (Livingston and Callinan, 2015; Sahker and Arndt, 2017; Staudt et al., 2019). In general, the process of diagnosing hazardous alcohol drinking (HAD) is based on self-reported data and is thereby vulnerable to socially acceptable responding and recall bias (Davis et al., 2010; Greenfield and Kerr, 2008). These factors may compromise the detection of individuals with HAD that might increase the risk of SB and hinder its prevention strategies.

Such reporting discrepancies might be addressed by the use of biomarkers that quantify alcohol consumption. One such putative biomarker of chronic alcohol consumption is phosphatidylethanol (PEth) (Varga and Alling, 2002), which is a relatively new tool being studied for detection and approximate quantification of a person's use of alcohol in security, medical, and legal environments (Ulwelling and Smith, 2018). PEth represents a group of phospholipids which are formed in cell membranes only if alcohol is present in its environment, and its level in erythrocytes is directly proportional to both ethanol concentration and duration of ethanol exposure (Gnann et al., 2012; Kobayashi and Kanfer, 1987). Since there are no enzymatic systems in human erythrocytes that can break down PEth, it accumulates with each alcohol use in relation to recency, quantity, and duration of alcohol use, reflecting a "history" of drinking (Schröck et al., 2017a). PEth can be detected three to four weeks after a single high dose of alcohol intake (above 6 standard units of alcohol per intake) and after continuous and long-term 'safe' use (up to 2 standard units of alcohol per day) (Schröck et al., 2017a).

Given the aforementioned evidence, PEth might prove to be an interesting tool to detect HAD in individuals experiencing suicidal crisis. However, there is a lack of studies investigating it in individuals with and without SA. The present study aimed to examine the difference in concentration of blood PEth in suicide attempters, depressed individuals, and individuals without psychiatric pathology. Our primary hypothesis was that the concentrations would be different between patients and control group. The secondary hypothesis was that the concentrations will differ significantly in all three groups.

2. Materials and methods

2.1. Study population

This case-control observational multi-centric study was conducted during the period between June 2020 and June 2021 in two major hospitals in Vilnius city. Data were analyzed between June and December of 2021. A total of 156 individuals were recruited into three study groups of equal size during their contact with a healthcare provider on a first-come basis, irrespective of their age or sex. A total of 156 individuals were recruited into three study groups of equal size. General study inclusion criteria were: (1) adult age, and (2) capacity to give informed consent. General exclusion criteria were: (1) currently suffering from a mental illness other than MDD, (2) currently suffering from a serious somatic illness that requires intensive treatment, (3) the person does not understand the national language, (4) deprived of liberty or reduced legal capacity as determined by a court, and (5) people who are unable to understand and sign the informed consent form.

To compare suicide attempters (cases) to both depressed and healthy controls, we recruited patients in three groups of equal size, with additional inclusion and exclusion criteria. (1) Suicide attempters (N = 51) were individuals who were admitted to the Toxicology Intensive Care Unit of the Republican Vilnius University Hospital after a SA, irrespective of the type of SA and clinical diagnosis, their additional inclusion criteria was a recent SA (within up to 48 h before the evaluation); (2) Major depressive disorder (MDD) without SA group (N = 52) were recruited at the Vilnius City Mental Health Center and Vilnius University Hospital Santaros Klinikos Center of Neurology Department of Psychiatry, their additional inclusion criterion was the clinical diagnosis of MDD, and additional exclusion criterion was a lifetime history of SA; (3) Non-psychiatric volunteers (N = 52) were individuals with no psychiatric diagnosis or SA history who visited the Center of Family Medicine of Vilnius University Hospital Santaros Klinikos for a prophylactic medical check-up, their additional exclusion criteria were SA history, a psychiatric diagnosis and the presence of significant suicidal ideation or depressive symptomatology during the inclusion interview. All other illnesses were allowed, but mostly it was healthy volunteers or local medical staff.

Interviews were collected during routine care, there were no incentives or invitations to participate. If the patient agreed, they signed informed consent and were included.

All the blood follow samples were taken at the same time – 09:00 in the morning. Drinking history was obtained by asking whether patients were drinking or not (binary question), and if yes, how much it was in international units during the week. In relationship means either married or in long term relationship (>2 years) with the same partner.

2.2. Measures

All study participants were interviewed regarding their psychiatric history by a trained clinical professional, who also administered the Hamilton Depression Rating Scale (HDRS-17) (HAMILTON, 1967). HDRS-17 is a 17-item interviewer-administered measure of the depressive symptom severity over the past week. It is one of the most commonly used instruments in psychiatry research to establish and quantify the severity of depression. Cut offs for this scale are as follows:

Not depressed: 0–7; Mild (subthreshold): 8–13; Moderate (mild): 14–18; Severe (moderate): 19–22; Very severe (severe): >23. In addition, study participants filled in the Beck Scale for Suicide Ideation (BSS) (Beck et al., 1988), which is a 19-item self-report instrument for detecting and measuring the current intensity of attitudes, behaviours, and plans to attempt suicide over the past week. The scale contained 19 items rated on a scale from 0 to 2, allowing scores between 0 and 38. It allows not only to establish whether there is a suicidal ideation or not, but also to evaluate its severity and to follow the dynamics with treatment. Score over 9 was proposed as threshold to indicate a significant risk for SA (Cochrane-Brink et al., 2000). They were also asked about alcohol use (yes or no question whether one considers him/herself a drinker or not). Demographics, information about patient smoking status, laboratory tests, and other psychiatric diagnoses were collected retrospectively from medical documentation. Blood samples were collected from all study participants by a trained nurse, and C-reactive protein (CRP) concentration was measured according to the standard hospital's protocol.

2.3. Blood PEth analysis

EDTA blood PEth concentrations were measured by the in-house liquid chromatography-tandem mass spectrometry (LC-MS/MS) method (Jones et al., 2011).

Chemicals and materials (solvents, reagents, and internal standards)

Phosphatidylethanol 16:0/18:1 (PEth), >99% was purchased from Avanti Polar Lipids, Inc. Phosphatidylethanol-d5 16:0/18:1 (PEth-d5), 1000 μg/ml solution in $CHCl_3$ was purchased from Chiron. Ammonium acetate (NH_4OAc), ≥99.0%; acetonitrile (ACN), ≥99.9%; methanol (MeOH), ≥99.8%, 2-propanol (IPA), ≥99.5% were purchased from Sigma-Aldrich. Deionized H_2O was produced in-house with a Milli-Q water system from Millipore (Molsheim, France). 1000 μg/ml stock solution of PEth was prepared by dissolving the purchased PEth standard in Methanol. Working solutions of PEth (10 μg/ml and 1 μg/ml) for calibration curve were prepared by successive dilution of stock solution with MeOH. 1 μg/ml working internal standard solution of PEth-d5 was prepared by several successive dilutions of purchased 1000 μg/ml standard solution of PEth-d5 with MeOH.

2.4. Sample preparation

Measurement of PEth was performed within 5 days after blood sample delivery to the laboratory. 100 μl of EDTA blood samples were transferred into 1.5 ml microcentrifuge tubes. A volume of 20 μl of internal standard working solution (1 μg/ml) and 400 μl of methanol was added. The tubes were capped, vortex-mixed for 10 s and shaken on an orbital shaker for 60 min at 400 rpm. After extraction, the solute was centrifuged at 14,000 rpm (5 min). After centrifugation, 200 μl of supernatant in 1.5 ml autosampler vials was diluted with 800 μl of mobile phase A.

2.5. Instrumentation

Chromatographic separation was performed on Shimadzu UHPLC system, including binary pump (LC-30AD), autosampler (SIL-30AC) and column oven (CTO-20AC), connected to LCMS-8060 mass spectrometer (Shimadzu), controlled by LabSolutions 1.2 software (Shimadzu). YMC-Triart C4 column (100 × 3.0 mm i.d., 1.9 μm) was used for chromatographic separation. Column temperature was maintained at 40 °C. The injection volume was 10 μL. Mobile phase A consisted of 20% 2 mM NH_4OAc/H_2O and 80% ACN (v/v). Mobile phase B consisted of 100% IPA. Separation was performed using 7 min gradient elution: 0 min–0.5 min, 90% A; 0.5 min to 3.0min, 90% to 0% A linear; 3.0min to 4.0 min, 0% A; 4.0 min–4.5 min, 0% A to 90% A linear; 4.5 min to 7.0min, 90% A. The mobile phase flow rate was 0.50 ml/min. Retention time of PEth was 2.13min, for PEth-d5 was 2.13min. Mass spectrometric detection was performed in ESI negative MRM mode, with interface voltage of −3000 V and a interface temperature 350 °C, DL temperature 270 °C, Heat Block temperature 400 °C, Heating gas flow 10 l/min, Nebulizing gas flow 3 l/min, Drying gas flow 10 l/min by measuring following transitions: PEth - 701.5/255.45 as a quantifier, 701.5/281.5 as a qualifier; PEth-d5 - 706.5/281.45 as a quantifier, 706.5/255.45 as a qualifier.

2.6. Method validation

Calibration curve was constructed using spiked whole EDTA blood. The procedure of preparation of spiked samples was as follows: venous blood from a male volunteer who abstained from alcohol consumption for five years, was collected into vacuum tubes with a lithium heparin anticoagulant. Aliquots of 100 μl of blood were spiked with relevant amounts of working solutions of PEth, in order to get the following concentrations of PEth: 0, 2.5, 7.5, 15, 30, 60, 120, 240, 480 ng/ml. Nine-point calibration curves of PEth were measured twice on several different days. Correlation coefficients for calibration curves were >0.998. Precision and accuracy were investigated by preparing blood samples, spiked with different PEth concentrations: 0 ng/ml, 5 ng/ml, 10 ng/ml, 20 ng/ml, 40 ng/ml, 80 ng/ml, 160 ng/ml. Limit of detection (LOD; lowest quantity of a substance that can be detected) was set at 3.2 ng/ml, and the limit of quantitation (LOQ; lowest quantity of the analyte that can be reliably quantified) was set at 9.6 ng/ml, as calculated using ANOVA. Selectivity was determined by testing 10 blank EDTA blood samples drawn from people who abstained from alcohol consumption more than 3 months, in order to test for potential interference of PEth signals with endogenous matrix components or metabolites. No interference was detected. Carry-over was examined by testing spiked blood samples with 5000 ng/ml PEth. No carry-over was detected.

Ethics approval

All procedures were in accordance with the Helsinki declaration and its later amendments or comparable ethical standards and adhered to Good Clinical Practice guidelines. All participants provided written informed consent. The study was approved by the Vilnius Regional Biomedical Research Ethics Committee of Lithuania (permission number: No.2020/9-1266-747).

2.7. Statistical analysis

Prior to the main analyses, raw data were tested for normality distribution of residuals, outliers, errors and plausibility. Continuous data were assessed for normality and homogeneity with the Shapiro-Wilk, Levene's tests, and visual inspection of probability plots. Kruskal-Wallis H test with post-hoc Bonferroni correction was used to compare linear measures between suicide attempters, MDD, and individuals without psychiatric diagnosis and SA. We then applied several multinomial logistic and linear regression models in order to see the predictive value of the PEth, after checking the distribution of residuals and collinearity. There was no collinearity, VIF was never more than 10. Differences between 3 groups have been calculated using ANOVA method. We also used a nonparametric Kendall's tau correlation coefficient (Xu et al., 2013) to measure the group differences.

The level of statistical significance was set at $p < .05$, two-sided. For family-wise comparisons, the Benjamini-Hochberg False Discovery Rate (FDR) procedure (Benjamini and Hochberg, 1995) was applied, with a threshold set at $q < 0.05$. Data were analyzed using the SPSS version 24 (IBM, Armonk, NY, USA).

3. Results

In the present study, the mean participant age was 38.24 ± 15.85 years. 69.2% (N = 108) of participants were females, which corresponds

to generally observed higher rates of MDD and SA in females than males in Europe (Hawton, 2000; Whiteford et al., 2013). A set of tests (χ^2 and non-parametric Kruskall Wallis) were used to establish the differences between the three groups (See Table 1). We observed no differences in sex and relationship status (See Table 1). Significant group differences were observed regarding the educational attainment and income, with the control group having the longest education and highest income, followed by the MDD group, and the suicide attempters' group having the shortest education and lowest monthly income. There was a trend for a difference when interpreting the age of the three groups, suicide attempters being the oldest, followed by the depressed group, and the control group, which did not reach the level of statistical significance. As expected, antidepressant use and HDRS-17 scores were the highest in MDD patients and lowest in healthy volunteers, while BSS score was the highest in suicide attempters and the lowest in healthy volunteers. We also observed that CRP levels were the highest in suicide attempters, followed by patients with MDD and the control group (Table 1, Fig. 3).

We have found both binary (detectable versus undetectable) and continuous differences in PEth concentrations with regard to the study group. 70.58% of suicide attempters had detectable PEth concentration, with 232,54 ± 394,01 ng/ml on average in all attempters, and 330.40 ± 433.93 ng/ml in those with detectable values. In MDD patients, these numbers decreased to 42.31%, 58,39 ± 135,82 ng/ml, and 139.14 ± 181.56. Meanwhile, with 60.37% detectability, the individuals in the control group were in-between suicide attempters and patients with MDD, but continuous concentrations were the lowest in this group - 24,45 ± 70,83 ng/ml in all individuals, and 42.53 ± 317.52 ng/ml in those with detectable PEth values. Differences in $\log_e$ PEth values between all group members are presented in Fig. 1. Differences in raw PEth values in individuals with detectable PEth values are presented in Fig. 2.

Post-hoc tests showed that not only suicide attempters had significantly higher PEth concentrations than the control group ($\chi^2 = 3.28$, p = .007), but also, significantly higher than the MDD group ($\chi^2 = 2.70$, p = .001). There was no difference between the MDD and the control groups ($\chi^2 = 0.60$, p = .55).

To test the robustness of our results, we assessed whether PEth concentration is associated to other potentially important factors that have been previously associated with SA, suicidal ideation or MDD, notably, sex, age, smoking, antidepressant use, and a low-grade inflammation marker CRP. We found that men had significantly higher PEth levels than females (155.74 ± 309.61 ng/ml vs 81.86 ± 227.50 ng/ml, U = 2055, p = .032), but the group differences between SA, MDD and control groups remained significant in both sexes. Dot plot figure is presented in Figs. 4 and 5. Smoking was also significantly associated with higher PEth levels (162.73 ± 324.05 ng/ml vs 81.36 ± 225.39 ng/ml, U = 1660, p = .002). Meanwhile, PEth levels were significantly lower in suicide attempters that used antidepressants compared to attempters without antidepressant use (132.88 ± 267.74

Table 1
Sociodemographic, clinical and PEth comparisons between three groups.

	Total (N = 156) Mean ± SD or N (%)	Depression (N = 52) Mean ± SD or N (%)	SA (N = 51) Mean ± SD or N (%)	Controls (N = 53) Mean ± SD or N (%)	χ^2 (df)	*Kruskal-Wallis* χ^2	p-value	Post-hoc comparison	p-value for post-hoc
Sociodemographic and clinical characteristics									
Sex, female	108 (69.2%)	39 (75.0%)	34 (66.7%)	35 (66.0%)	1.22	..	.542	..	..
Age, years	38.24 ± 15.85	39.75 ± 13.24	41.63 ± 19.80	33.49 ± 12.77	..	5.77	.056	..	..
Education, years	15.63 ± 3.75	15.10 ± 3.21	12.6 ± 2.43	18.80 ± 2.66	..	64.13	<.001**[a]	Control > depression Control > SA Depression > SA	<.001** <.001** .002*
HDRS-17 score	13.18 ± 8.45	19.71 ± 5.14	16.25 ± 5.23	3.81 ± 4.37	..	96.49	<.001**[a]	SA > control Depression > control	<.001** <.001*
BSS score	5.12 ± 6.61	4.81 ± 6.40	9.92 ± 6.73	0.81 ± 2.25	..	73.53	<.001**[a]	SA > control SA > depression Depression > control	<.001** <.001** .001*
In a relationship	59 (44.7%)	19 (36.5%)	12 (40.0%)	28 (53.8%)	3.20 (2)	..	.20	..	..
Antidepressant use	80 (50.3%)	50 (96.2%)	30 (58.8%)	0 (0%)	98.86 (2)	..	<.001**[a]	Depression > control SA > control Depression > SA	<.001** <.001**
Smoker, current	44 (28.76%)	22 (42.3%)	19 (37.3%)	3 (5.77%)	20.47 (2)	..	<.001**[a]	Depression > control SA > control	<.001** <.001**
Self-reported alcohol use	92 (65.71%)	31 (60.78%)	19 (52.78%)	42 (79.25%)	7.53 (2)	..	.023*	Control > SA	.008*
Blood PEth and serum C-reactive protein concentrations									
PEth binary: found	77 (49.4%)	22 (42.3%)	36 (70.6%)	32 (60.4%)	..	8.67	.013*	SA > depression	.004*
PEth continuous ng/ml	103.80 ± 257.16	58.39 ± 135.82	232.54 ± 394,01	24.45 ± 70.83	..	12.23	.002*[a]	SA > depression SA > control	.003* .021*
PEth: males only ng/ml	155.74 ± 309.61	96.57 ± 146.50	349.24 ± 447.13	15.73 ± 28.87	..	11.50	.003*[a]	SA > control	.002*
PEth: females only ng/ml	81.86 ± 227.50	46.30 ± 131.37	175.22 ± 356.93	30.78 ± 84.20	..	6.29	.043*	SA > depression	.045*
C-reactive protein mg/l	5.00 ± 15.80	3.00 ± 4.03	12.46 ± 28.43	1.40 ± 2.36	..	14.61	.001*[a]	SA > control Depression > control	.001* .034*

Abbreviations: BSS, Beck Scale for Suicidal Ideation, self-report (Beck et al., 1988); df, degree of freedom; PEth, phosphatidylethanol; HDRS-17, Hamilton Depression Rating Scale (HAMILTON, 1967); SA, suicide attempter; SD, standard deviation.
*Significant at p < .05; **Significant at p < .001.
[a] Significant at Benjamini-Hochberg False Discovery Rate (Benjamini and Hochberg, 1995) corrected p-value <.003 (q < 0.05) for family-wise group comparisons.
All continuous variables were non-normally distributed, and the non-parametric Kruskal-Wallis test coupled with the post-hoc comparisons with Bonferroni correction to determine pairwise group differences.

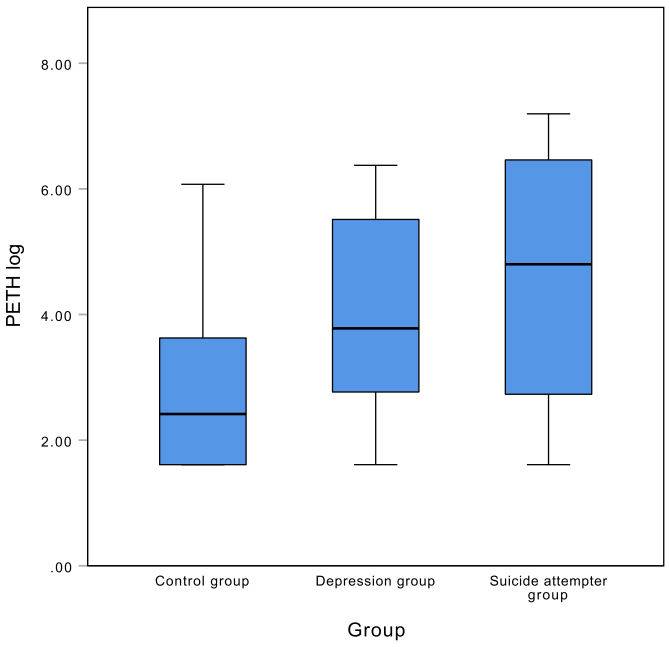

Fig. 1. PEth log_e values in all participants in the three groups.

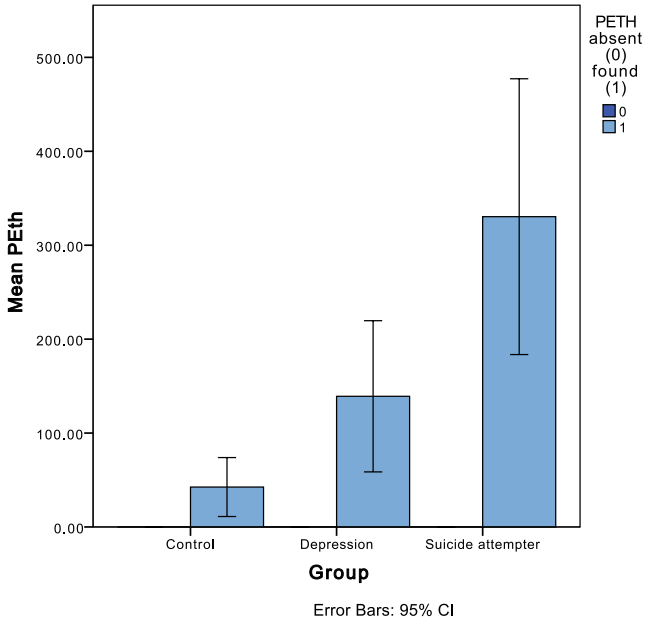

Fig. 2. Peth (ng/ml) raw mean values in three groups only in participants with detectable PEth values.

ng/ml vs. 376.57 ± 496.70, ng/ml, U = 177, p = .007). The difference regarding the antidepressant use was not significant in the MDD group (p = .85). Finally, we observed a positive correlation between the CRP and PEth levels (Kendall's tau = 0.13, p = .044) (see Fig. 6).

Next, we compared whether alcohol use self-report was indicative of chronic alcohol consumption, and observed a positive association (self-reported users (N = 92) 117.98 ± 257.92 ng/ml vs. self-reported non-users (N = 48) 14.45 ± 41.52 ng/ml; U = 1138, p < .001). A *t*-test further confirmed this finding, with an overall medium effect size between self-reported alcohol use and PEth concentration, d = 0.49, p < .001. As a post-hoc analysis, we then analyzed which individuals underreported their alcohol use based on the mismatch between the PEth concentration and the self-report use, and found that 8 of 43 cases

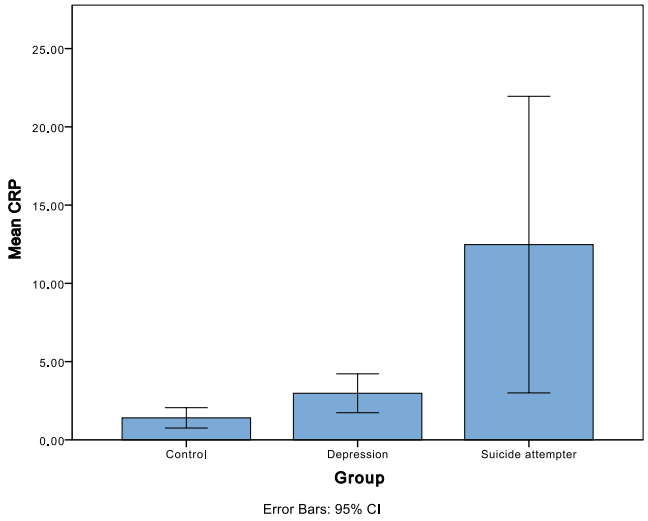

Fig. 3. CRP (mg/l) raw mean values in three groups.

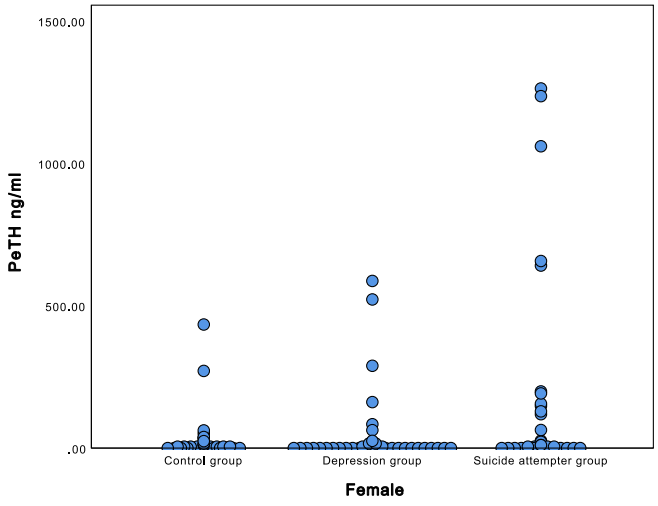

Fig. 4. Dot-plot figure on individual PEth values between 3 groups, females.

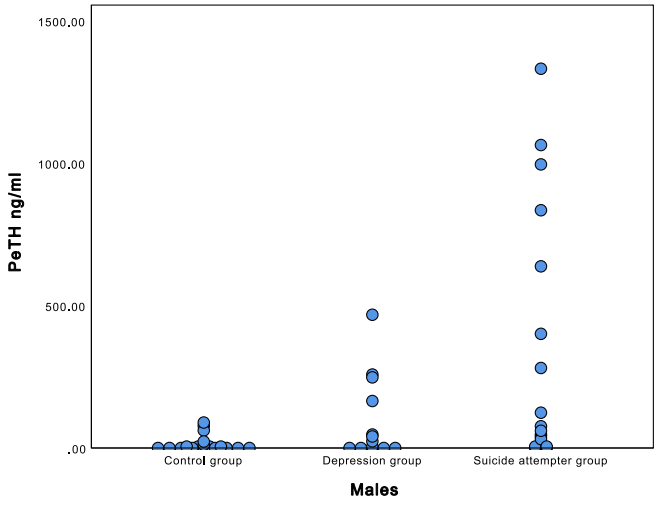

Fig. 5. Dot figure on individual PEth values between 3 groups, males.

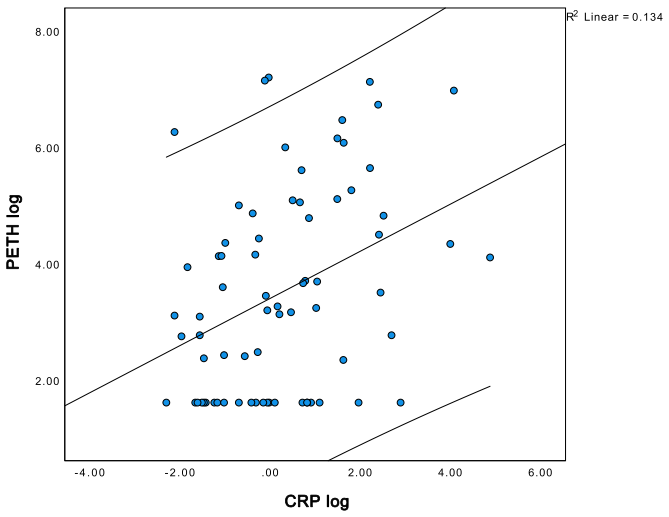

Fig. 6. Correlation between logarithmized PeTH concentrations and logarithmized CRP concentrations, with 95% CI.

(18.6%) in suicide attempters' group and 10.41% in MDD group might have underreported their use, while it was 0 in MDD group ($\chi^2 = 8.37$, p = .015).

3.1. Multivariate analyses

Several multivariate models were applied to examine the independence of the association between PEth concentration and SA history. In the unadjusted multinomial logistic regression model (overall model $\chi^2 = 21.59$, $p < .001$), which considered the control group as the reference group, PEth levels were not predictive of patients belonging to the MDD group (OR = 1.003, p = .18), but were significantly predictive of patients belonging to the suicide attempters' group (OR = 1.006, p = .01), suggesting that for every increase in PEth blood levels by 1 ng/ml, the risk of belonging to the suicide attempters', rather than control group increased by 0.6%. In a model adjusted for sex, education and age, PEth remained significantly higher in suicide attempters compared to controls (p = .012, aOR = 1.006).

In a model adjusted for CRP levels and antidepressant use (known to affect inflammation levels), the PEth was no longer statistically significantly associated with SA (p = .09). These results are detailed in Table 2.

Finally, we performed a linear regression model to see whether PEth could predict the HDRS-17 and BSS scores. In the whole sample, the PEth was not significantly predictive of HDRS-17 and BSS scores. However, when we performed regressions separately for each group, PEth was significantly negatively associated with HDRS-17 ($R^2 = 0.10$, F (1; 49) = 5.97, p = .018) and BSS scores ($R^2 = 0.11$, F (1,49) = 6.22, p = .016) in suicide attempters' group. No such differences were observed in MDD and control groups. We then performed adjustments. In the new regression table, adjusted for CRP, sex, age, smoking - PEth was still significant. When we have changed PEth with the subjective question to the patient whether he/she uses alcohol, that variable was not significant. Also, when we removed PEth, CRP by itself was not statistically significant.

See Table 3 for further detail.

4. Discussion

To our knowledge, the present study provides the first evidence that PEth, an objective measure of HAD, is elevated in recent suicide attempters, as compared to both – individuals with no psychiatric history and MDD patients. Our findings extend on multiple previous studies that have established a strong connection between harmful alcohol use and SB (Darvishi et al., 2015) and found elevated blood alcohol concentration in suicide descendants (Chitty et al., 2021), suggesting that PEth might serve as a useful test to objectify the level of recent and medium-term alcohol exposure in individuals presenting with a suicidal crisis or are at risk for it.

In general, our results are in line with previous studies that have reported a positive association between self-reported alcohol consumption and PEth values (Jørgenrud et al., 2021; Schröck et al., 2017b), suggesting that most individuals do indeed disclose alcohol use. Yet, a recent study has reported that PEth concentration was a more informative phenotype than self-reported data for revealing epigenetic mechanisms associated with HAD (Liang et al., 2021). Notably, we have observed significant group differences in the present study, with significantly higher rates of underreporting of recent alcohol consumption, as demonstrated by the discrepancy between PEth concentration and self-report measure, in suicide attempters compared to both - patients with MDD and the control group, with MDD patients showing the lowest rates of underreporting. While trust in patients is primordial in clinical settings, several reasons might explain the underreporting in patients after SA: lower confidence in medical professionals, the treatment being often not self-initiated, memory bias related to confusion caused by medication intoxication during the SA. PEth use might therefore prove to be superior to self-report only in these patients when assessing HAD.

Another interesting finding that emerged from the present study was a negative correlation between PEth concentrations and both BSS and HDRS-17 scores, which is in contrast to the generally accepted notion that HAD and chronic alcohol use are associated with depressive symptomatology and higher rates of SI (Darvishi et al., 2015; Strid et al.,

Table 2
Multinomial logistic regression model, with PEth levels (ng/ml) as a depended variable and belonging to a group as an independent variable.

	Variable	B	SE	Wald	df	p-value	OR	95% CI
Model 1[a]								
	Depression	.003	.002	1.81	1	.18	1.003	0.999–1.007
	Suicide attempt	.006	.002	6.637	1	.01[d]	1.006	1.001–1.010
Model 2[b]								
	Depression	.002	.002	1.127	1	.288	1.002	.998–1.006
	Suicide attempt	.006	.002	6.379	1	.012[d]	1.006	1.001–1.010
Model 3[c]								
	Depression	.003	.004	.675	1	.411	1.003	.996–1.011
	Suicide attempt	.006	.003	2.805	1	.094	1.006	.999–1.012

Reference value was the control group in all models.
Abbreviationsdf, degrees of freedom; CI, Confidence Interval; OR, Odds Ratio; SE, Standard Error.
[a] Crude association.
[b] Adjusted for education, sex and age.
[c] Adjusted for antidepressant use and CRP levels.
[d] p-value <.05 (two-sided).

Table 3
Multivariate linear regression models with PEth and self-reported alcohol use predicting the severity of suicidal ideation and depression in suicide attempters.

Dependent variable: Beck Scale for Suicide Ideation						
Model 1 – with PEth		Unstandardized Coefficients: B	Std. Error	Standardized Coefficients: Beta	T score	p-value
	(Constant)	13.968	3.631		3.846	<.001**
	Sex	.676	2.475	.047	.273	.787
	Age	−.077	.057	−.217	−1.365	.183
	CRP	−.019	.048	−.066	−.398	.693
	PEth	**−.008**	**.003**	**−.421**	**−2.671**	**.012***
	Smoking	3.886	2.320	.264	1.675	.104

Model 2 – with self-reported alcohol use		Unstandardized Coefficients: B	Std. Error	Standardized Coefficients: Beta	T score	p-value
	(Constant)	20.610	5.169		3.988	<.001**
	Sex	1.764	3.152	.129	.560	.583
	Age	−.177	.088	−.449	−2.015	.060
	CRP	−.020	.124	−.036	−.158	.877
	Self-reported alcohol use	−1.587	3.241	−.116	−.490	.631
	Smoking	−.775	3.128	−.057	−.248	.807

Dependent Variable: Hamilton Depression Rating Scale score						
Model 1 – with PEth		Unstandardized Coefficients: B	Std. Error	Standardized Coefficients: Beta	T score	p-value
1	(Constant)	18.934	2.890		6.551	<.001**
	Sex	−.063	1.970	−.006	−.032	.975
	Age	−.050	.045*	−.180	−1.107	.277
	CRP	−.035	.038*	−.154	−.912	.369
	PEth	**−.006**	**.002***	**−.448**	**−2.789**	**.009***
	Smoking	1.649	1.847	.143	.893	.379

Model 2 – with self-reported alcohol use		Unstandardized Coefficients: B	Std. Error	Standardized Coefficients: Beta	T score	p-value
	(Constant)	22.005	3.524		6.244	<.001**
	Sex	.879	2.149	.093	.409	.688
	Age	−.071	.060	−.263	−1.187	.252
	CRP	−.113	.084	−.302	−1.336	.199
	Self-reported alcohol use	−1.266	2.210	−.135	−.573	.574
	Smoking	−1.463	2.132	−.158	−.686	.502

Analyses in the table are in suicide attempters group (N = 51).
Abbreviations: CRP, C-reactive protein; PEth, phosphatidylethanol.
*Significant at $p < .05$, two-sided; **Significant at $p < .001$, two-sided.

2018). PEth levels were also lower in suicide attempters that were undergoing treatment with antidepressant medications. Several possible explanations exist for this finding. First, people in suicidal crisis have impaired decision-making capacity (Sastre-Buades et al., 2021) and insight (de Assis da Silva et al., 2017), and might not be able to accurately evaluate their own condition. In our study, this notion is supported by underreporting of alcohol consumption. A possible direct neurotoxic effect of alcohol on the brain, most notably the prefrontal cortex (Collins and Neafsey, 2016; Topiwala et al., 2017), might underlie this effect. The prefrontal cortex is functionally impaired in people with alcohol addiction (Park et al., 2010). Both rodent and human studies show that chronic alcohol exposure is associated with altered synaptic plasticity in the prefrontal cortex (Kroener et al., 2012; Loheswaran et al., 2017), hindering their decision-making capacity. Not to be forgotten is the possibility of lack of thiamine (vitamin B1) in the brain of chronic alcohol consumers and resulting in Wernicke-Korsakoff encephalopathy (Martin et al., 2003).

Regarding the depressive symptomatology, some suicide attempts might be associated with such factors as impulsivity and impaired inhibition rather than depressive symptomatology, suggesting a putatively independent from depression pathway to SA. Indeed, recent studies point to the heterogeneity of SB, with some cases having marked impulsivity, childhood adversity, and family history patterns, whereas others are more linked to MDD (Lengvenyte et al., 2021a; Oquendo and Baca-Garcia, 2014). While we did not measure impulsivity, it is interesting to hypothesize that suicide attempters with high PEth levels might belong to the former group. In addition, two-thirds of study participants in the present study were females. This corresponds to female proportion in other studies from cohorts of individuals with MDD and anxiety disorders (Fried et al., 2019) and in suicide attempters (Courtet et al., 2015). It also corresponds to generally reported elevated female to male ratio in MDD and SA in Europe (Hawton, 2000; Whiteford et al., 2013). While we adjusted our major analyses for sex, univariate comparisons showed that males had significantly higher PEth levels than females in all groups. However, while females make more SA and are generally more likely to be treated for MDD, males are more likely to die by suicide (Hawton, 2000), suggesting that markers linked to SA, such as PEth, might not necessarily perform well in suicide prediction.

Finally, alcohol might have had a short-term antidepressant effect. Acute alcohol consumption has been linked to increased opioid levels (Wolfe et al., 2016), suggesting that suicide attempters might self-medicate their psychological pain, a major component in the pathophysiology of SB (Conejero et al., 2018), with alcohol.

Aside from impulsivity and self-medication, several other possible explanations exist MDD patients and suicide attempters had higher levels of PEth. Heavy alcohol is known to cause a whole-body low-grade inflammation (González-Reimers, 2014). It has been shown that CRP (a marker of systemic inflammation) is directly associated with the level of alcohol consumption (Oliveira et al., 2010), which was also the case in the present study, as we observed a correlation between CRP levels and PEth concentrations. We report that suicide attempters had the highest CRP levels, followed by MDD patients and the control group. This is in line with previous studies showing an association between CRP and depression and further above and beyond association between CRP and SA in depressed patients (Chamberlain et al., 2019; Gibbs et al., 2016; Miola et al., 2021). In addition, CRP has also been associated with depressive symptoms in healthy volunteers (Khan et al., 2020), suggesting it is a state rather than a trait marker. It is worth mentioning that the difference in CRP levels between suicide attempters and patients with MDD was almost 4-fold in the present study, while it was further

2-fold between MDD patients and the control group. Also, when the association between PEth and the patient group (SA, depression or healthy volunteer) in multinomial logistic regression was adjusted for CRP and antidepressant use (which are known also to impact CRP levels and other inflammatory parameters (Tynan et al., 2012)), PEth was no longer significantly higher in suicide attempters, suggesting that the association between PEth and SA may be at least partly mediated by systemic inflammation caused by heavy alcohol consumption (Oliveira et al., 2010). In the linear regression model in suicide attempters, PEth was a significant predictor of BSS and HDRS-17 scores. In the further exploratory analysis, after adjustments for age, sex, CRP and smoking it was still significant predictor of beforementioned scores. Also, when we changed the PEth variable to the binary self-reported alcohol use, the association was not statistically significant.

In sum, PEth could be more useful for prediction than asking about alcohol use to patient directly, and it is even better predictor than CRP (not significant in our model), which is has been previously repeatedly associated with SA (Courtet et al., 2015; Miola et al., 2021). This reinforces the possible value of PEth screening in people at risk of SA.

4.1. Strenghts and limitations

This is the first study to show elevated PEth levels in individuals with recent SA and MDD with current depressive episode. While the results will have to be replicated, PEth is easily dosable and might be used in clinical practice as an easy-access screening tool. Another important finding is that PEth levels helped to differentiate suicide attempters from the MDD group. This could be useful in clinical practice because there are very few valid SA prognostic markers, and currently it is impossible to predict SA and suicide (Kessler et al., 2020). Due to an array of reasons, such as stigma, denial, and lack of treatment compliance, patients do not always disclose SA, depression symptom severity, and their drinking habits in full detail. PEth could provide the caretakers with an additional level of objectified evidence about the severity of the alcohol use problem at hand.

Nevertheless, results need to be interpreted with caution. First, the observational cross-sectional design does not allow us to infer causality. We also cannot make any conclusions regarding death by suicide. Second, we did not collect data on SA methods, severity and lethality. Violent SA has been associated with higher CRP levels (Aguglia et al., 2019). On the other hand, blood alcohol concentration has been reported to be higher in suicide victims by non-lethal means (Zupanc et al., 2013). Therefore, the complex association between SA methods, PEth and inflammation should be further investigated. Although we defined recent SA as up to 48 h before the evaluation, we did not register the exact time between SA and blood collection within this timeframe, precluding a more granular analysis regarding the PEth levels and the immediate recency of the SA. This study was also a convenience sample, possibly suffering from voluntary response bias, and some heavy drinkers might have been inclined to refuse to participate.

Furthermore, the study was underpowered to measure differences in our secondary outcomes reliably. Also, only patients sufficiently severe to be treated in hospital settings were included in SA and depression groups, possibly causing a severity bias. This adult sample is also not generalizable to children and adolescents, in whom drinking habits have been associated with suicidal behaviours, and for whom alcohol and drug testing have been proposed as a part of the evaluation of the suicidal crisis due to a possible underreporting (Aseltine et al., 2009). We also did not examine other factors that might be associated with PEth levels, such as the body mass index, race, haemoglobin levels, and liver fibrosis (Hahn et al., 2021), as well as some factors that could be related to SA, such as psychiatric comorbidity. Deep clinical phenotyping is warranted in future studies. It should also be noted that the PEth levels reflect alcohol exposure, which is related to SA but is highly unlikely to be a stand-out biomarker. PEth capability to detect and predict SA risk, especially in combination with factors associated with SA, remains to be established before its application in clinical practice.

5. Conclusions

Though preliminary, our study shows for the first time that recent SA is associated with significantly increased PEth levels in blood compared to patients with MDD and individuals without psychiatric complaints. MDD patients, in turn, have higher PEth levels than individuals without psychiatric complaints. Suicide attempters were also more likely to underreport their recent alcohol consumption in self-report measures, suggesting the particular interest of objective testing in this patient population. These findings indicate that PEth might be a helpful tool to complement the clinical assessment of individuals at risk of SA. Nevertheless, further well-designed prospective studies are needed to confirm the external validity of our findings through replication in an independent cohort and assess the actual utility of measuring PEth compared to classical self-report measures studied in suicide risk evaluation, prediction and prevention.

Author contribution

RS and AL and wrote and revised the manuscript. LZ and RB made critical improvements and analyzed the data. ED, EM, MM, LV collected the data. JS, TP and AK analyzed Peth levels. AU and SL provided their expert opinions, LA and AL supervised the whole process.

Funding source

Vilnius University, Faculty of Medicine.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors want to thank participants and healthcare personnel who provided services to patients.

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ORIGINAL ARTICLE

Antibiotic Prophylaxis in Infants with Grade III, IV, or V Vesicoureteral Reflux

W. Morello, E. Baskin, A. Jankauskiene, F. Yalcinkaya, A. Zurowska, G. Puccio, J. Serafinelli, A. La Manna, G. Krzemień, M. Pennesi, C. La Scola, F. Becherucci, M. Brugnara, S. Yuksel, D. Mekahli, R. Chimenz, D. De Palma, P. Zucchetta, D. Vajauskas, D. Drozdz, M. Szczepanska, S. Caliskan, J. Lombet, D.G. Minoli, S. Guarino, K. Gulleroglu, D. Ruzgiene, A. Szmigielska, E. Barbi, Z.B. Ozcakar, A. Kranz, A. Pasini, M. Materassi, S. De Rechter, G. Ariceta, L.T. Weber, P. Marzuillo, I. Alberici, K. Taranta-Janusz, A. Caldas Afonso, M. Tkaczyk, M. Català, J.E. Cabrera Sevilla, O. Mehls, F. Schaefer, and G. Montini, for the PREDICT Study Group*

ABSTRACT

BACKGROUND

The efficacy of continuous antibiotic prophylaxis in preventing urinary tract infection (UTI) in infants with grade III, IV, or V vesicoureteral reflux is controversial.

METHODS

In this investigator-initiated, randomized, open-label trial performed in 39 European centers, we randomly assigned infants 1 to 5 months of age with grade III, IV, or V vesicoureteral reflux and no previous UTIs to receive continuous antibiotic prophylaxis (prophylaxis group) or no treatment (untreated group) for 24 months. The primary outcome was the occurrence of the first UTI during the trial period. Secondary outcomes included new kidney scarring and the estimated glomerular filtration rate (GFR) at 24 months.

RESULTS

A total of 292 participants underwent randomization (146 per group). Approximately 75% of the participants were male; the median age was 3 months, and 235 participants (80.5%) had grade IV or V vesicoureteral reflux. In the intention-to-treat analysis, a first UTI occurred in 31 participants (21.2%) in the prophylaxis group and in 52 participants (35.6%) in the untreated group (hazard ratio, 0.55; 95% confidence interval [CI], 0.35 to 0.86; P=0.008); the number needed to treat for 2 years to prevent one UTI was 7 children (95% CI, 4 to 29). Among untreated participants, 64.4% had no UTI during the trial. The incidence of new kidney scars and the estimated GFR at 24 months did not differ substantially between the two groups. Pseudomonas species, other non–*Escherichia coli* organisms, and antibiotic resistance were more common in UTI isolates obtained from participants in the prophylaxis group than in isolates obtained from those in the untreated group. Serious adverse events were similar in the two groups.

CONCLUSIONS

In infants with grade III, IV, or V vesicoureteral reflux and no previous UTIs, continuous antibiotic prophylaxis provided a small but significant benefit in preventing a first UTI despite an increased occurrence of non–*E. coli* organisms and antibiotic resistance. (Funded by the Italian Ministry of Health and others; PREDICT ClinicalTrials.gov number, NCT02021006; EudraCT number, 2013-000309-21.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Dr. Montini can be contacted at giovanni.montini@unimi.it or at the Pediatric Nephrology, Dialysis, and Transplant Unit, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico di Milano, Via della Commenda 9, 20122 Milan, Italy.

*The members of the PREDICT study group are listed in the Supplementary Appendix, available at NEJM.org.

This article was published on September 12, 2023, at NEJM.org.

N Engl J Med 2023;389:987-97.
DOI: 10.1056/NEJMoa2300161

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Continuous antibiotic prophylaxis is routinely used in infants with grade III, IV, or V vesicoureteral reflux. The aim is to prevent urinary tract infections (UTI) and potential long-term sequelae associated with kidney scarring.[1-3]

Previous pediatric trials on UTI prevention have focused mainly on children with absent or low-grade vesicoureteral reflux, predominantly in girls with associated bladder or bowel dysfunction.[3-6] In the large Randomized Intervention for Children with Vesicoureteral Reflux (RIVUR) trial, in which young children underwent randomization after a first or second UTI, more than 90% were female, with a wide age range, and 80% had grade II or III vesicoureteral reflux.[7] Although continuous antibiotic prophylaxis was effective in preventing recurrent UTIs,[7-9] there was no apparent effect on kidney scarring,[2,7] a finding that raises questions about the clinical significance of this intervention.[10] The role of primary continuous antibiotic prophylaxis in infants with grade III, IV, or V vesicoureteral reflux (grades that have often been associated with congenital kidney damage) and no previous UTI is unclear.

The use of continuous antibiotic prophylaxis in infants must be weighed against the potential emergence of multidrug-resistant isolates[11,12] and adverse effects on gut microbiota.[13-15] Both are important public health issues.

We now report results from the Antibiotic Prophylaxis and Renal Damage in Congenital Abnormalities of the Kidney and Urinary Tract (PREDICT) trial. In the trial, we assessed whether continuous antibiotic prophylaxis would be effective in preventing the occurrence of a first symptomatic UTI and would avert secondary kidney damage in infants with grade III, IV, or V vesicoureteral reflux and no previous UTIs.

METHODS

TRIAL DESIGN AND OVERSIGHT

This investigator-initiated, phase 3, multicenter, prospective, randomized, open-label trial was performed in 39 European centers within the ESCAPE Network.[16] Institutional review boards at all the participating sites approved the trial. The authors vouch for the completeness and accuracy of the data and for the fidelity of the trial to the protocol, available with the full text of this article at NEJM.org. There was no agreement regarding the confidentiality of the data between the sponsors and the authors.

TRIAL POPULATION

The inclusion criteria were an age of 1 to 5 months; vesicoureteral reflux with a grade of III, IV, or V as assessed by voiding cystourethrography or voiding ultrasonography[17]; a gestational age of 35 weeks or more; and an estimated glomerular filtration rate (GFR) of more than 15 ml per minute per 1.73 m^2 of body-surface area (2009 Schwartz formula).[18] Infants with previous UTI, posterior urethral valves, neurogenic bladder, or ureteropelvic-junction or ureterovesical-junction obstruction were excluded. At baseline, all the infants were assessed by kidney and bladder ultrasonography, a dimercaptosuccinic acid (DMSA) scan, measurement of the serum creatinine level, and urinalysis.

STRATIFICATION, RANDOMIZATION, AND INTERVENTION

Eligible children were randomly assigned (in a 1:1 ratio) to receive continuous antibiotic prophylaxis (prophylaxis group) or no treatment (untreated group) for 2 years. The randomization was stratified according to the presence or absence of kidney parenchymal damage (Table S1 in the Supplementary Appendix, available at NEJM.org).

The antibiotic choice for continuous antibiotic prophylaxis was left to the site investigators, according to local *Escherichia coli* resistance patterns. Treatment options included nitrofurantoin at a dose of 1.5 mg per kilogram of body weight per day, amoxicillin–clavulanate at a dose of 15 mg per kilogram per day (expressed in amoxicillin-equivalent units), cefixime at a dose of 2 mg per kilogram per day, and trimethoprim–sulfamethoxazole at a dose of 2.5 mg per kilogram per day (expressed in trimethoprim-equivalent units and preferably prescribed after 3 months of life).

Prophylaxis was administered as a single daily dose, with the option of changing the antibiotic in the event that unacceptable side effects occurred. After a first UTI, continuous antibiotic prophylaxis could be modified among one of the four possible options, according to the antibiotic sensitivities, in order to overcome resistance.

FOLLOW-UP PROCEDURES

Participants were monitored at baseline and at 4, 8, 12, 18, and 24 months. In the event of symp-

tomatic UTIs or adverse events, additional visits occurred (Table S2). Adherence to continuous antibiotic prophylaxis was evaluated at each visit with diaries filled out by families.

Participants were assessed by means of ultrasonography, DMSA scan, and voiding cystourethrography or voiding ultrasonography at baseline and 2 years. Images were uploaded to a central database, and DMSA scans with centrally available images were reevaluated in a blinded fashion by three of the authors (nuclear medicine physicians at different institutions) to assess possible focal uptake defects (number and position), global reduction of isotope uptake, and possible kidney asymmetry. In case of disagreement, images were reassessed and discussed and a final joint decision was made.

OUTCOMES

The primary outcome was the occurrence of a first symptomatic UTI during the 24-month trial. Secondary outcomes were the total number of UTIs during the 24-month trial, new kidney scars, the estimated GFR at 24 months, causative organisms and antibiotic resistance in UTI isolates, and serious adverse events.

TRIAL DEFINITIONS

Symptomatic UTI was defined as the concomitant presence of acute symptoms (e.g., fever of ≥38°C, unwell appearance, irritability, or loss of appetite), leukocyte esterase or nitrites on urinalysis, and a positive urine culture. A positive urine culture was defined as any growth from a suprapubic bladder aspirate, the growth of a single organism to at least 10,000 colony-forming units (CFU) per milliliter from a catheter sample, or the growth of a single organism to at least 100,000 CFU per milliliter from a midstream voided sample. Bagged specimens were not allowed. Local DMSA defects on a renal scan were defined as focal areas of reduced tracer uptake that were associated with loss of contours or cortical thinning.[19]

STATISTICAL ANALYSIS

On the basis of previous trials,[20] we anticipated a risk of symptomatic UTIs of approximately 35% among untreated participants during the 2 years of study. For sample-size calculation, we considered a between-group difference of 15 percentage points (with a risk of approximately 20% among participants receiving continuous antibiotic prophylaxis) to be clinically important. With an alpha error of 0.05, a power of 90%, and an estimated dropout rate of 25%, the sample-size calculation was 436 participants (218 per group).

A prespecified interim analysis was conducted 1 year after the randomization of 200 participants (November 27, 2018). We conducted the analysis in 116 participants with complete follow-up (60 in the prophylaxis group and 56 in the untreated group), using an alpha of 0.01 and the outcome at 24 months. The criteria for early interruption for efficacy or futility were not met (z-test for proportions; one-sided P value of 0.33; $z=0.43$), and no safety issues were observed. The steering committee agreed to continue the trial and to reduce the power to 80%, in view of the steady accrual rate of 50 participants per year, which resulted in a required sample size of 218 participants (109 per group), with 290 participants (145 per group) to be recruited under the assumption of an unchanged 25% dropout rate.

In the efficacy assessment, all the participants who underwent randomization were included in the intention-to-treat analysis. The first UTI was considered to be the event. Data for participants who did not have a symptomatic UTI were censored either at 24 months (those with the final visit) or at the time that they were lost to follow-up. A time-to-event Cox regression model was used to include data from all the participants who underwent randomization, in order to avoid potential bias. Several sensitivity analyses were performed to detect possible bias in the intention-to-treat analysis (Table S3). Cox and logistic-regression models included analysis for possible confounding and effect modifiers.

RESULTS

PARTICIPANT CHARACTERISTICS

From October 2013 through January 2020, a total of 867 infants were screened and 292 underwent randomization, 146 to each group. Reasons for screening failure were an age of more than 5 months (26.8%), absent or low-grade vesicoureteral reflux (35.0%), previous UTI (32.9%), and a lack of parental consent (5.4%) (Fig. S1). Participants were recruited from 39 European centers in Italy (42.8%), Turkey (24.0%), Poland (17.5%), Lithuania (7.9%), Belgium (4.8%), and other European countries (3.0%).

The 292 enrolled participants included 227 boys

(77.7%), of whom 5 (2.2%) had been circumcised for religious reasons. At recruitment, the median age was 3.4 months (interquartile range, 2.4 to 4.1), 80.5% of the participants had grade IV or V vesicoureteral reflux, and 48.3% had bilateral vesicoureteral reflux (Table 1). Focal congenital defects were identified in 83 participants (28.4%) on the baseline DMSA scan. In addition, DMSA abnormalities, including diffuse decreased uptake and unbalanced kidney function, were detected in 154 participants (52.7%). Congenital kidney defects were not associated with the grade of vesicoureteral reflux. Ten participants had a solitary kidney. Before enrollment, 150 participants (51.4%) had received previous antibiotic prophylaxis. The clinical characteristics of the participants at baseline did not differ substantially between the two groups.

In the prophylaxis group, amoxicillin–clavulanate was the antibiotic used most often for prophylaxis (72 participants [49.3%]). That was followed by trimethoprim–sulfamethoxazole (35 participants [24.0%]), nitrofurantoin (24 [16.4%]), and cefixime (15 [10.3%]).

PRIMARY OUTCOME

A first symptomatic UTI occurred in 31 participants (21.2%) in the prophylaxis group and in 52 participants (35.6%) in the untreated group (hazard ratio, 0.55; 95% confidence interval [CI], 0.35 to 0.86; P=0.008 by log rank test). The percentage of febrile UTIs was similar in the two groups (25 of 31 [81%] in the prophylaxis group and 41 of 52 [79%] in the untreated group; rate ratio, 1.02; 95% CI, 0.82 to 1.28). Urine cultures were collected by midstream voided samples in 49 of 83 cases (59%) and by catheter in 34 of 83 cases (41%). The time to the first UTI was 6.4 months in the prophylaxis group and 5.2 months in the untreated group (mean difference, 1.2 months; 95% CI, –1.3 to 3.6). Figure 1 shows the UTI-free survival among the 292 participants according to trial group. UTI-free survival was uninfluenced by the type of antibiotic used (Fig. S2).

All baseline data were checked in the Cox regression models to verify their possible association with the outcome (Table S4). Sex was a strong predictor of first UTIs, with male participants having a hazard ratio of 0.46 (95% CI, 0.29 to 0.73). When we controlled for sex, continuous antibiotic prophylaxis was still associated with the outcome (hazard ratio, 0.51; 95% CI, 0.33 to 0.80) (Table S5). The number needed to treat was 7 (95% CI, 4 to 29) to prevent one UTI within 2 years.

A total of 49 participants (16.8%) were lost to follow-up, 29 in the prophylaxis group and 20 in the untreated group. Of these, 13 (27%) had a first UTI before being lost to follow-up (5 in the prophylaxis group and 8 in the untreated group). Results of the sensitivity analyses were consistent with the results of the primary intention-to-treat analysis. The effect of treatment was similar, with similar effect sizes. The hazard ratio, rate ratio, and odds ratio in the prophylaxis group ranged from 0.53 to 0.64, with similar 95% confidence intervals (Table S3).

In the subgroup analysis of the treatment effect in all six combinations of participant sex and grade of vesicoureteral reflux, continuous antibiotic prophylaxis was associated with the outcome in female participants with grade IV or V vesicoureteral reflux. In male participants, a weak association was observed only in those with grade IV vesicoureteral reflux (Fig. 2).

SECONDARY OUTCOMES

Total UTIs

A total of 139 symptomatic UTIs occurred during the 24-month trial period, 60 in the prophylaxis group and 79 in the untreated group (rate ratio, 0.76; 95% CI, 0.59 to 0.97). Although participants with 1 or 2 UTIs were more common in the untreated group, participants with 3 or more UTIs were more common in the prophylaxis group (Fig. S3). The percentage of symptomatic UTIs that resulted in hospitalization was similar in the two groups (16 of 60 [27%] in the prophylaxis group and 24 of 79 [30%] in the untreated group; rate ratio, 0.88; 95% CI, 0.51 to 1.50). Most of the first symptomatic UTIs (50 of 82 cases with available data [61%]) were treated with oral antibiotics. The percentage of UTIs that were treated intravenously was similar in the two groups (13 of 30 [43%] in the prophylaxis group and 19 of 52 [37%] in the untreated group; rate ratio, 1.19; 95% CI, 0.69 to 2.04).

New Kidney Scars

DMSA data at both baseline and 24 months were available for 201 participants (83.7% of those with complete follow-up). At baseline, before any UTI, congenital kidney defects were present in 83 infants, 43 in the prophylaxis group and 40 in the

Table 1. Clinical and Demographic Characteristics of Enrolled Infants at Baseline.*

Characteristic	Prophylaxis Group (N=146)	Untreated Group (N=146)	Total (N=292)
Male sex — no. (%)	110 (75.3)	117 (80.1)	227 (77.7)
White race — no. (%)	140 (95.9)	141 (96.6)	281 (96.2)
Median age (IQR) — mo	3.4 (2.4–4.1)	3.4 (2.3–4.1)	3.4 (2.4–4.1)
Coexisting condition at screening — no. (%)†	11 (7.5)	9 (6.2)	20 (6.8)
Parental consanguinity — no./total no. (%)	6/141 (4.3)	4/143 (2.8)	10/284 (3.5)
Previous antibiotic prophylaxis — no. (%)‡	69 (47.3)	81 (55.5)	150 (51.4)
Phimosis in male infants — no./total no. (%)§	20/110 (18.2)	31/117 (26.5)	51/227 (22.5)
Abnormality on prenatal ultrasonography — no./total no. (%)	107/141 (75.9)	111/139 (79.9)	218/280 (77.9)
Hydronephrosis on prenatal ultrasonography — no./total no. (%)	95/141 (67.4)	105/139 (75.5)	200/280 (71.4)
Grade of vesicoureteral reflux — no. (%)¶			
III	29 (19.9)	28 (19.2)	57 (19.5)
IV	60 (41.1)	59 (40.4)	119 (40.8)
V	57 (39.0)	59 (40.4)	116 (39.7)
Bilateral vesicoureteral reflux — no. (%)¶	65 (44.5)	76 (52.1)	141 (48.3)
Split function on DMSA scan — no. (%)			
Absent: 0 to 10%	13 (8.9)	11 (7.5)	24 (8.2)
Reduced: >10 to <45%	72 (49.3)	76 (52.1)	148 (50.7)
Normal: ≥45%	61 (41.8)	59 (40.4)	120 (41.1)
Overall DMSA abnormalities — no. (%)‖	80 (54.8)	74 (50.7)	154 (52.7)
Bilateral overall DMSA abnormalities — no. (%)	17 (11.6)	15 (10.3)	32 (11.0)
Local defects on DMSA scan — no. (%)**	43 (29.5)	40 (27.4)	83 (28.4)
Bilateral local defects on DMSA scan — no. (%)	5 (3.4)	7 (4.8)	12 (4.1)
Solitary kidney — no. (%)	2 (1.4)	8 (5.5)	10 (3.4)
Hyperechogenicity in one kidney on baseline ultrasonography — no. (%)	24 (16.4)	22 (15.1)	46 (15.8)
Pelvic dilatation ≥5 mm on baseline ultrasonography — no. (%)	108 (74.0)	99 (67.8)	207 (70.9)
Bilateral pelvic dilatation ≥5 mm on baseline ultrasonography — no. (%)	41 (28.1)	33 (22.6)	74 (25.3)
Ureteric dilatation on baseline ultrasonography — no. (%)	77 (52.7)	64 (43.8)	141 (48.3)
Bilateral ureteric dilatation on baseline ultrasonography — no. (%)	25 (17.1)	18 (12.3)	43 (14.7)
Bladder-wall irregularity on baseline ultrasonography — no. (%)	12 (8.2)	8 (5.5)	20 (6.8)
Systolic blood pressure >90th percentile — no./total no. (%)	7/126 (5.6)	8/125 (6.4)	15/251 (6.0)
Diastolic blood pressure >90th percentile — no./total no. (%)	27/123 (22.0)	29/122 (23.8)	56/245 (22.9)
Median estimated GFR (IQR) — ml/min/1.73 m^2††	81.3 (59.1–109.9)	83.2 (62.1–111.2)	82.6 (61.0–110.6)

* Participants in the prophylaxis group were assigned to receive continuous antibiotic prophylaxis for 24 months. Participants in the untreated group were assigned to receive no prophylaxis. DMSA denotes dimercaptosuccinic acid, GFR glomerular filtration rate, and IQR interquartile range.

† Coexisting conditions at screening were defined as separate medical conditions, other than vesicoureteral reflux, that were simultaneously present in the enrolled participants (syndromic features, intrauterine growth retardation, cardiac defects, gastrointestinal defects, genital abnormalities, hematologic disorders, lung disease, endocrinopathies, and maternal diabetes).

‡ Previous antibiotic prophylaxis was defined as the use of antibiotic prophylaxis before enrollment.

§ Phimosis was defined as the inability to retract the foreskin with bulging or ballooning during urination.

¶ This characteristic was assessed by voiding cystourethrography in 283 participants and by voiding ultrasonography in 9 participants.

‖ Overall DMSA abnormalities were defined as the presence of local defects, diffuse defects, or both on a DMSA scan.

** The number of local defects ranged from one to three per participant.

†† The estimated glomerular filtration rate (GFR) was calculated according to the 2009 Schwartz formula.[18]

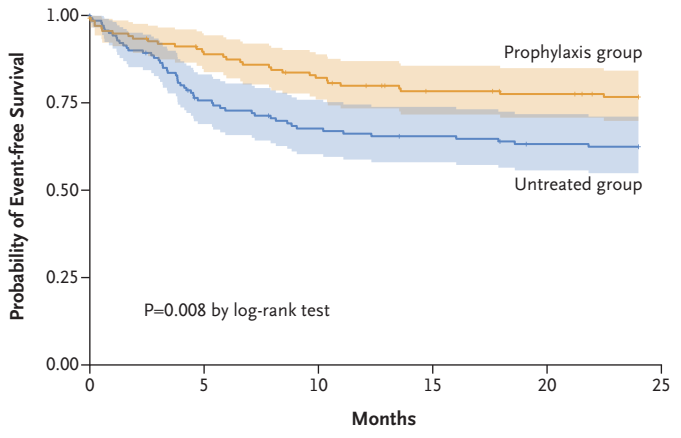

Figure 1. Urinary Tract Infection (UTI)–free Survival during the 24-Month Trial.

Participants in the prophylaxis group were assigned to receive continuous antibiotic prophylaxis for 24 months. Participants in the untreated group were assigned to receive no prophylaxis. The shaded areas represent 95% pointwise confidence intervals. Confidence intervals were not adjusted for multiplicity and should not be used to infer definitive treatment effects.

untreated group (Table 1). At 24 months, new kidney defects were identified in 21 participants in the prophylaxis group and 17 participants in the untreated group (rate ratio, 1.22; 95% CI, 0.69 to 2.18). The number of new lesions ranged from one to five per child. The change in the number of focal defects from baseline to 24 months was similar in the two groups in our sample (Fig. S4).

The number of new lesions was independent of the occurrence of UTIs during the trial period. New defects were identified in 27 of 144 participants (18.8%) who had no UTI and in 11 of 57 (19%) who had at least one UTI (rate ratio, 0.97; 95% CI, 0.52 to 1.83). Participants with kidney defects at baseline were not at greater risk for UTIs during the 24-month follow-up in our sample. These results were confirmed in the blinded evaluation of 108 participants with centrally available images (Fig. S5).

Estimated GFR

Data on the estimated GFR at both baseline and 24 months were available for 228 participants (93.8% of those with complete follow-up). The estimated GFR was similar in the two groups at baseline (Table 1) and at 24 months (mean, 112.3 ml per minute per 1.73 m^2 in the prophylaxis group and 109.5 ml per minute per 1.73 m^2 in the untreated group; mean difference, 2.8 ml per minute per 1.73 m^2; 95% CI, −4.8 to 10.3). Serum creatinine values at 24 months were also similar in the two groups (228 participants: mean, 0.37 in the prophylaxis group and 0.37 in the untreated group; mean difference, 0.00; 95% CI, −0.04 to 0.04). The estimated GFR at 24 months was similar in participants with a UTI and those without a UTI (mean, 105.8 ml per minute per 1.73 m^2 and 112.8 ml per minute per 1.73 m^2, respectively; mean difference, –7.0 ml per minute per 1.73 m^2; 95% CI, –18.0 to 1.0).

UTI Isolates and Antibiotic Resistance

Isolates differed according to trial group, with *E. coli*, klebsiella species, and proteus species more commonly found in untreated participants, whereas all pseudomonas infections and an increased percentage of non–*E. coli* isolates were observed in participants who received continuous antibiotic prophylaxis (Table 2). Resistance to at least two first-line antibiotics (including amoxicillin, amoxicillin–clavulanate, and second- or third-generation cephalosporin) was present in 16 of 31 isolates (52%) in the prophylaxis group and 9 of 52 isolates (17%) in the untreated group, with a rate ratio of 2.98 (95% CI, 1.50 to 5.92) (Table S6).

Serious Adverse Events

Serious adverse events were reported in 9 of 146 participants (6.2%) in the prophylaxis group and 6 of 146 participants (4.1%) in the untreated group (P=0.43). No events of special interest were reported. Table 3 summarizes all serious adverse events that occurred during the trial, according to trial group.

REPRESENTATIVENESS OF THE TRIAL POPULATION

Our population was representative of infants with high-grade vesicoureteral reflux with associated kidney hypodysplasia and no previous UTI, with the expected male-to-female ratio. The percentage of non-White participants was small (3.8%), owing both to the pooled European population and the lower incidence of vesicoureteral reflux among Black persons than among non-Black persons. The results may not be applicable to children who receive a diagnosis of vesicoureteral reflux after a first UTI or to older female children (Table S7).

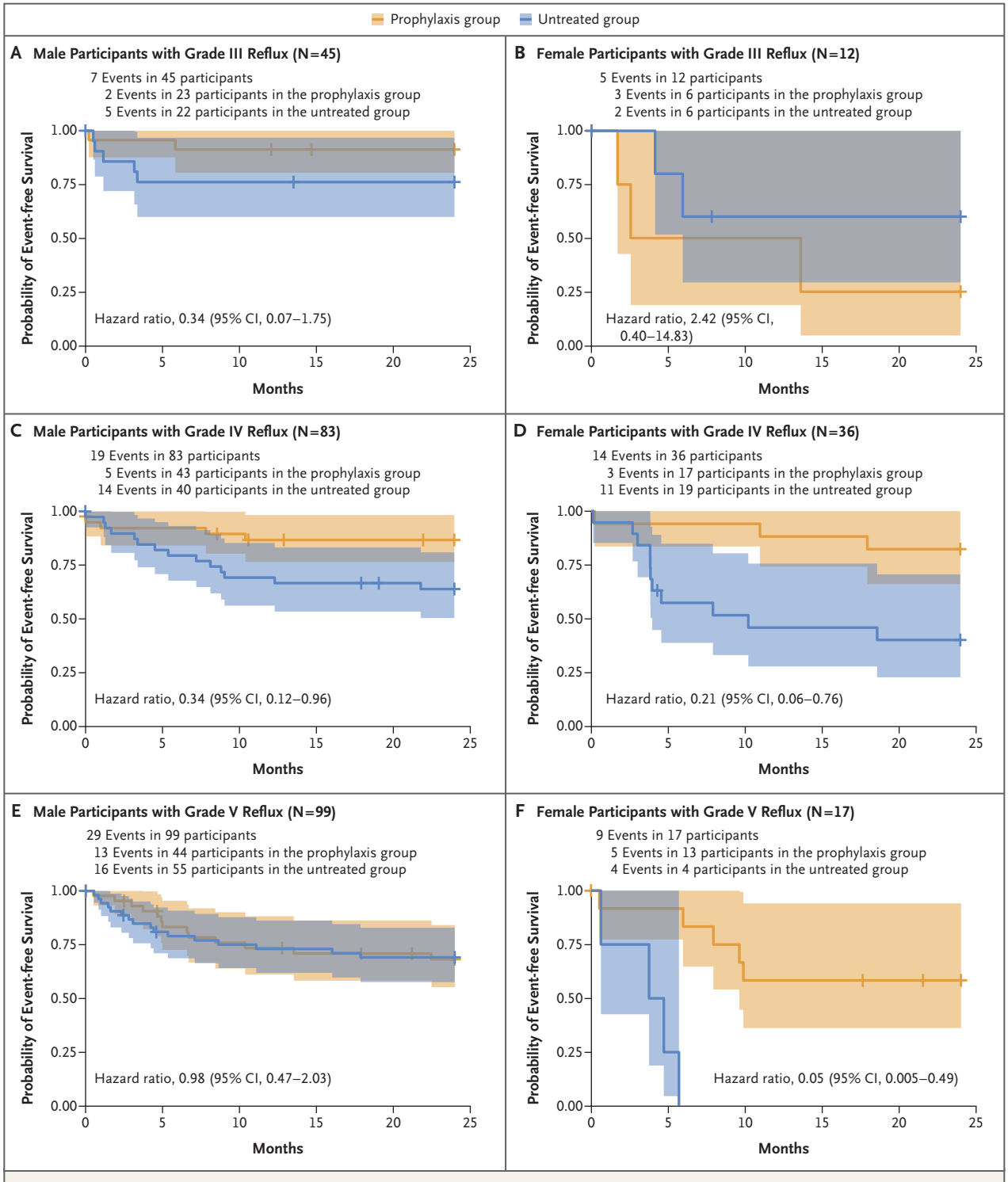

Figure 2. Subgroup Analysis According to Participant Sex and Grade of Vesicoureteral Reflux.

Shown is the probability of survival free from a first UTI. Shaded areas represent 95% pointwise confidence intervals. Confidence intervals were not adjusted for multiplicity and should not be used to infer definitive treatment effects.

Table 2. Urinary Tract Infection (UTI) Isolates.

Isolate	Prophylaxis Group	Untreated Group
	number (percent)	
Total	31 (100)	52 (100)
Candida albicans	1 (3)	0
Citrobacter species	1 (3)	1 (2)
Escherichia coli	13 (42)	29 (56)
Enterobacter cloacae	2 (6)	1 (2)
Enterococcus faecalis or *E. faecium*	2 (6)	2 (4)
Klebsiella species	5 (16)	13 (25)
Morganella morganii	0	1 (2)
Proteus species	0	4 (8)
Pseudomonas species	6 (19)	0
Undetermined	1 (3)	1 (2)

DISCUSSION

In this multicenter, randomized, controlled trial, we assessed and quantified the efficacy of continuous antibiotic prophylaxis administered before the occurrence of any UTI in infants with grade III, IV, or V vesicoureteral reflux. The incidence of a first symptomatic UTI was lower by 14.4 percentage points among participants who received continuous antibiotic prophylaxis for 24 months than among untreated participants. However, a UTI did not develop in 64.4% of the untreated participants; thus, the number needed to treat was seven children for 2 years to prevent one UTI. Continuous antibiotic prophylaxis was not associated with the occurrence of new kidney scars or with the estimated GFR at 24 months, but an increased occurrence of pseudomonas and other non–*E. coli* organisms as well as increased antibiotic resistance were observed. No substantial difference in the percentage of UTIs that resulted in hospitalization was noted.

Our trial cohort of young (median age, 3.4 months), predominantly male (77.7%) infants with grade III, IV, or V vesicoureteral reflux (grades that are often associated with innate kidney damage) and no previous UTI represents a population of children with congenital abnormalities of the kidney and urinary tract who are at increased risk for chronic kidney disease.[1] The cohort differs from the cohorts of earlier trials, which consisted predominantly of older female children who had already had a UTI, with low-grade or absent vesicoureteral reflux, normal kidneys, and a favorable long-term outcome.

In the Prevention of Recurrent Urinary Tract Infection in Children with Vesicoureteric Reflux and Normal Renal Tracts (PRIVENT) trial involving 576 children under the age of 18 years who had had a previous UTI, the incidence of UTI recurrence was lower by 6 percentage points among those who received continuous antibiotic prophylaxis than among those who received placebo. However, 41% of the children in that trial who underwent urinary tract imaging had no vesicoureteral reflux.[21] The Swedish Reflux Trial, in which 203 children 1 year of age with grade III or IV vesicoureteral reflux were randomly assigned to one of three groups (continuous antibiotic prophylaxis, endoscopic reflux correction, or clinical observation), showed lower incidences of UTI recurrence and kidney damage among girls receiving continuous antibiotic prophylaxis, with no differences in boys.[22] The RIVUR trial[7] evaluated the efficacy of trimethoprim–sulfamethoxazole in 607 children (92% female) 2 to 72 months of age with grade I to IV vesicoureteral reflux (92% with grade I, II, or III reflux) and normal kidneys, after a first or second UTI. The risk of UTI recurrence was lower by 11.9 percentage points in the prophylaxis group than in the placebo group, and the number needed to treat was 8 children for 2 years to prevent one UTI (a finding similar to that in our trial).

Our results show a significant effect of continuous antibiotic prophylaxis in preventing the occurrence of a first UTI, but no substantial differences were observed in the appearance of new scar formation, the estimated GFR at 24 months, or hospitalizations for UTIs. These findings support the results of previous trials and a meta-analysis of seven trials involving 1076 children with vesicoureteral reflux,[2] in which continuous antibiotic prophylaxis did not prevent kidney scarring in otherwise healthy children. Furthermore, in the current trial, the occurrence of new kidney defects was not associated with the presence or absence of UTIs.

The small but significant effect of continuous antibiotic prophylaxis in preventing UTI, with no evident effect on kidney scarring in the sample enrolled, must be weighed against the develop-

ment of antibiotic resistance[4,11] and change in the developmental trajectory of eubiotic gut microbiota.[13,14] In our trial, UTIs in participants in the prophylaxis group were associated with a greater number of non–*E. coli* isolates, including six infections with pseudomonas species, and increased antibiotic resistance. The increased resistance of bacterial isolates to antibiotics in the prophylaxis group probably reflects the selection of resistant strains. This occurrence should not be underestimated, in light of the major health concerns associated with the emergence of multidrug-resistant bacteria.[23-25]

Our results indicate that only one third of untreated infants with grade III, IV, or V vesicoureteral reflux will have a UTI during their first 2 years of life. The 53 female participants with grade IV or V reflux showed a benefit from continuous antibiotic prophylaxis, in accordance with the results of the Swedish Reflux Trial,[22] whereas in the largest subgroup, the 99 male participants with grade V reflux, continuous antibiotic prophylaxis was not associated with any relevant benefit. For these reasons, we believe that the routine use of continuous antibiotic prophylaxis is not justified and should be considered only in female patients with grade IV or V vesicoureteral reflux or to prevent reinfections that may occur after a first UTI.

In our trial cohort, all the infants had congenital abnormalities of the kidney and urinary tract, as shown by prenatal or postnatal ultrasonography. In the absence of symptomatic UTI, our data cast doubt on the need for invasive procedures for the detection of vesicoureteral reflux, particularly voiding cystourethrography, when the result may not influence subsequent management. These findings suggest that further imaging should be reserved to rule out posterior urethral valves or ureterocele.

Our trial has several limitations. One is its open-label design, although the unequivocal and well-defined primary outcome may indicate a minimum of investigator or parent bias and expectations affecting results. The inclusion of participants from different European countries with different resistance patterns of *E. coli*[26] precluded the universal designation of a single antibiotic for UTI prophylaxis. Moreover, only a subset of DMSA images were available for central reading. More than 95% of our participants were White; that fact may affect applicability to infants of other racial backgrounds. Finally, our results cannot be generalized to children with previous UTI, who were excluded from the trial.

Table 3. Serious Adverse Events in the Intention-to-Treat Population.

Serious Adverse Event	Prophylaxis Group (N=146)	Untreated Group (N=146)
Total	9	6
Bronchiolitis*	0	3
Gastroenteritis*	1	2
Diarrhea*	2	0
Sepsis*	1	0
Pneumonia*	1	0
Fever*	1	0
Febrile seizure*	1	0
Sudden infant death syndrome	1	0
Cardiac surgery*	0	1
Implantation of prosthetic eye socket*	1	0

* The event was considered to be serious because it resulted in hospitalization or prolonged an existing hospitalization.

The strengths of the trial include a large, multicenter, diverse population of participants with similar characteristics in both groups. The trial retention rate was high, with low nonadherence and dropout rates. Furthermore, our trial succeeded in addressing the efficacy of continuous antibiotic prophylaxis as a therapeutic strategy in a real-life scenario, in which the antibiotic for continuous antibiotic prophylaxis was selected by physicians according to local patterns of bacterial resistance, thus providing results that we speculate can be generalized to all children with grade III, IV, or V vesicoureteral reflux.

Although our trial showed a numerical benefit of primary continuous antibiotic prophylaxis in young infants with vesicoureteral reflux without preceding UTI, the results are of doubtful clinical benefit and we believe do not support the routine use of continuous antibiotic prophylaxis in this population. A UTI did not develop in almost two thirds of the untreated participants, and the number needed to treat to prevent a UTI was 7, with a small difference in primary-outcome events; no apparent difference in kidney scarring, kidney function, or hospitalization for UTIs; and unfavorable effects on the spectrum of causative organisms and their resistance patterns.

In our trial, we found a small but significant benefit of primary continuous antibiotic prophylaxis in preventing a first UTI in young infants with vesicoureteral reflux and without preceding UTI.

The results were partially presented at the Belgian Pediatric Nephrology Symposium 2022, Brussels, December 13, 2022, and at the 33rd Congress of the European Society for Pediatric Urology, Lisbon, Portugal, April 19 to 22, 2023.

Supported by a grant from the Italian Ministry of Health (research project RF-2010-2308451) and by the Italian nonprofit organizations il Sogno di Stefano and Associazione per il Bambino Nefropatico. Drs. Morello, Jankauskiene, Zurowska, Becherucci, Mekahli, Drozdz, Ariceta, Weber, Caldas Afonso, Schaefer, and Montini are members of the European Rare Kidney Disease Reference Network.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

We thank Dr. Ian Hewitt for his invaluable assistance in the preparation of an earlier version of the manuscript.

APPENDIX

The authors' full names and academic degrees are as follows: William Morello, M.D., Ph.D., Esra Baskin, M.D., Augustina Jankauskiene, M.D., Fatos Yalcinkaya, M.D., Aleksandra Zurowska, M.D., Giuseppe Puccio, M.D., Jessica Serafinelli, M.D., Angela La Manna, M.D., Grażyna Krzemień, M.D., Marco Pennesi, M.D., Claudio La Scola, M.D., Ph.D., Francesca Becherucci, M.D., Milena Brugnara, M.D., Selcuk Yuksel, M.D., Djalila Mekahli, M.D., Ph.D., Roberto Chimenz, M.D., Diego De Palma, M.D., Pietro Zucchetta, M.D., Donatas Vajauskas, M.D., Dorota Drozdz, M.D., Maria Szczepanska, M.D., Salim Caliskan, M.D., Jacques Lombet, M.D., Dario G. Minoli, M.D., Stefano Guarino, M.D., Kaan Gulleroglu, M.D., Dovile Ruzgiene, M.D., Agnieszka Szmigielska, M.D., Egidio Barbi, M.D., Zeynep B. Ozcakar, M.D., Anna Kranz, M.D., Andrea Pasini, M.D., Marco Materassi, M.D., Stephanie De Rechter, M.D., Gema Ariceta, M.D., Lutz T. Weber, M.D., Pierluigi Marzuillo, M.D., Irene Alberici, M.D., Katarzyna Taranta-Janusz, M.D., Alberto Caldas Afonso, M.D., Marcin Tkaczyk, M.D., Margarita Català, M.D., Jose E. Cabrera Sevilla, M.D., Otto Mehls, M.D., Franz Schaefer, M.D., and Giovanni Montini, M.D.

The authors' affiliations are as follows: the Pediatric Nephrology, Dialysis, and Transplant Unit (W.M., G.P., J.S., G.M.) and the Pediatric Urology Unit (D.G.M.), Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, and the Department of Clinical Sciences and Community Health, University of Milan (G.M.), Milan, the Department of General and Specialized Surgery for Women and Children, Università degli Studi della Campania "Luigi Vanvitelli," Naples (A.L.M., S.G., P.M.), the Institute for Maternal and Child Health–IRCCS Burlo Garofolo (M.P., E. Barbi) and the Department of Medical, Surgical, and Health Sciences, University of Trieste (E. Barbi), Trieste, Pediatric Nephrology and Dialysis, Pediatric Unit, IRCCS Azienda Ospedaliero–Universitaria di Bologna, Bologna (C.L.S., A.P.), the Nephrology and Dialysis Unit, Meyer Children's Hospital IRCCS, Florence (F.B., M.M.), Pediatria C, Ospedale Donna Bambino, Verona (M.B.), the Pediatric Nephrology and Rheumatology Unit, Azienda Ospedaliera Universitaria Gaetano Martino, University of Messina, Messina (R.C.), the Nuclear Medicine Unit, Circolo Hospital and Macchi Foundation, Azienda Socio Sanitaria Territoriale Sette Laghi, Varese (D.D.P.), the Nuclear Medicine Unit, Department of Medicine, University Hospital of Padua (P.Z.), and the Pediatric Nephrology, Dialysis, and Transplantation Unit, Department of Women's and Children's Health, University of Padua (I.A.), Padua — all in Italy; the Department of Pediatric Nephrology, Başkent University Faculty of Medicine (E. Baskin, K.G.), and the Department of Pediatric Nephrology, Ankara University School of Medicine (F.Y., Z.B.O.), Ankara, the Department of Pediatric Nephrology and Pediatric Rheumatology, Pamukkale University School of Medicine, Denizli (S.Y.), and the Department of Pediatric Nephrology, Istanbul University–Cerrahpaşa, Cerrahpaşa School of Medicine, Istanbul (S.C.) — all in Turkey; the Pediatric Center, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius (A.J., D.R.), and the Department of Radiology, Medical Academy, Lithuanian University of Health Sciences Kauno Klinikos, Kaunas (D.V.) — both in Lithuania; the Department of Pediatrics, Nephrology, and Hypertension, Medical University of Gdansk, Gdansk (A.Z., A.K.), the Department of Pediatrics and Nephrology, Medical University of Warsaw, Warsaw (G.K., A.S.), the Department of Pediatric Nephrology and Hypertension, Chair of Pediatrics, Pediatric Institute, Jagiellonian University Medical College, Krakow (D.D.), the Department of Pediatrics, Faculty of Medical Sciences in Zabrze, Medical University of Silesia in Katowice, Katowice (M.S.), the Department of Pediatrics and Nephrology, Medical University of Bialystok, Bialystok (K.T.-J.), and the Department of Pediatrics, Immunology, and Nephrology, Polish Mother's Memorial Hospital Research Institute, Lodz (M.T.) — all in Poland; the Department of Pediatric Nephrology, University Hospitals Leuven (D.M., S.D.R.), and PKD Research Group, Laboratory of Ion Channel Research, Department of Cellular and Molecular Medicine, KU Leuven (D.M.), Leuven, and the Division of Nephrology, Department of Pediatrics, University Hospital Center of Liège, Liège (J.L.) — all in Belgium; the Department of Pediatric Nephrology, University Hospital Vall d'Hebron, and Universidad Autónoma de Barcelona, Hospital Vall d'Hebron (G.A.), and Pediatric Nephrology, Hospital General de Granollers, Universitat Internacional de Catalunya (M.C.), Barcelona, and Hospital General Universitario Santa Lucía, Cartagena (J.E.C.S.) — all in Spain; the University of Cologne, Faculty of Medicine and University Hospital Cologne, Children's and Adolescents' Hospital, Cologne (L.T.W.), and the Division of Pediatric Nephrology, Center for Pediatrics and Adolescent Medicine, Heidelberg University Hospital, Heidelberg (O.M., F.S.) — both in Germany; and the Division of Pediatric Nephrology, Centro Materno Infantil do Norte, Centro Hospitalar Universitário do Porto, Porto, Portugal (A.C.A.).

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Dokumento sudarytojas (-ai)	Medicinos fakultetas, tel. 2398700, faks. 2398705, M.K.Čiurlionio g. 21, LT-03101 Vilnius, el. p.: mf@mf.vu.lt
Dokumento pavadinimas (antraštė)	TARYBOS NUTARIMAS DĖL REIKŠMINGIAUSIŲ VILNIAUS UNIVERSITETO MEDICINOS FAKULTETO 2023 METŲ MOKSLO PASIEKIMŲ TEIKIMO VILNIAUS UNIVERSITETO MOKSLININKŲ SKATINIMO UŽ REIKŠMINGUS MOKSLO PASIEKIMUS ATRANKOS KONKURSUI
Dokumento registracijos data ir numeris	2024-02-28, (1.13 E) 150000-TPN-18
Adresatas	–
Dokumentą pasirašė	Profesorius Eugenijus Lesinskas
Veiksmo atlikimo data ir laikas	2024-02-28 09:31:57
Registratorius	Komunikacijos koordinatorė Indrė Mažeikienė
Veiksmo atlikimo data ir laikas	2024-02-28 09:43:07
Dokumento nuorašo atspausdinimo data ir jį atspausdinęs darbuotojas	2024-02-28 atspausdino Komunikacijos koordinatorė Indrė Mažeikienė

Nuorašas tikras
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2024-02-28