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Deficits in Alcohol Enforcement Legislation in Ireland: Bottomless Brunches

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Alcohol remains a clear and present danger to population health in Ireland. In response to this threat, the Irish Government passed the Public Health (Alcohol) Act, 2018. In addition to introducing Minimum Unit Pricing (MUP) and alcohol warning labels, this legislation also prohibits the selling of alcohol at a reduced price and the sale of alcohol in a manner likely to encourage alcohol consumption. However, this paper identifies numerous examples of 'bottomless brunches' in Ireland wherein unlimited alcohol is provided for a fixed price within a certain time period. A robust response from the Health Services Executive (HSE) and the Government is required to respond to alcohol as a Commercial Determinant of Health (CDoH).

The World Health Organization (WHO) has identified alcohol as an important Commercial Determinant of Health (CDoH) [1,2]. Alcohol is responsible for approximately 2.6 million deaths annually and constitutes more than 5% of the total global burden of disease and injury [3–5]. The significant adverse impacts of alcohol on health and well-being globally are mirrored in recent research from Ireland's Health Research Board (HRB) [6]. Recent evidence suggests that alcohol is the 8th leading cause of death in Ireland and is responsible for 8% of deaths [7].

In response to the damage wrought by alcohol, the Irish Government passed the Public Health (Alcohol) Act, 2018 (PHAA) [8]. This Act introduced several key measures, including Minimum Unit Pricing (MUP), and the impending commencement of mandatory alcohol warning labels. Although the Government has been criticised both for its deficits and for the slow enactment of the PHAA 2018 [9–11], it remains a significant piece of alcohol-control legislation.

Section 23 of the PHAA 2018 relates to the sale and supply of alcohol products (8). Among other prohibitions, it permits the Minister to make regulations prohibiting the supply and sale of alcohol at a reduced price. These regulations were subsequently provided for by the Public Health (Alcohol) Act 2018 (Sale and Supply of Alcohol Products) Regulations 2020, which came into operation on 11 January 2021 (see Table 1).

Table 1. Regulations 4, 5 and 6 of the Public Health (Alcohol) Act 2018 (Sale and Supply of Alcohol Products) Regulations 2020

4. A person shall not sell or supply, or cause to be sold or supplied, an alcohol product at a reduced price or free of charge to any person on the purchase by that person, or any other person, of - (a) one or more other alcohol products (whether of the same or a different kind), or (b) any other product or service.
5. A person shall not sell or supply, or cause to be sold or supplied, an alcohol product for a period of 3 days or less at a price less than that being charged for the alcohol product on the day before the commencement of the period concerned.
6. A person shall not advertise or promote, or cause to be advertised or promoted, the sale or supply of alcohol products in a manner specified in Regulation 4 or 5.

It is arguable that these public health regulations are being breached by the provision of so-called 'bottomless brunches', wherein, for a set fee, unlimited alcohol is provided alongside food for a set time period [12]. The drinks provided routinely include sparkling wine in the form of Prosecco, Mimosas, or Bellinis. The law prohibits the sale or supply of alcohol at a reduced price to any person on the purchase of any other product or service. The supply of unlimited alcohol is contingent upon the purchasing of food. As such, it can be argued that the sale and supply

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of alcohol in this fashion represents a violation of the Public Health (Alcohol) Act 2018 (Sale and Supply of Alcohol Products) Regulations 2020.

A cursory internet search by one of the authors lasting less than 4 hours identified 18 establishments offering bottomless brunches including unlimited alcohol in Dublin (n=5), Cork (n=5) Limerick (n=2), Galway (n=2), Kilkenny (n=1), Sligo (n=1), Tipperary (n=1), and Athlone (n=1).

Figure 1. Online Advert for a Dublin based Boozy Brunch

Figure 1 details an example advert for one such Bottomless Brunch, albeit described in this particular advert more explicitly as a 'Boozy Brunch'.

More detail on such bottomless brunches can be seen in the following web-based advert for another Dublin-based establishment:

> *For just €49.50 per person, you'll be treated to a delectable brunch main dish paired with an endless flow of cocktails, all within a relaxed 2-hour sitting…We are serving up epic flavours and unbeatable vibes alongside our classic boozy brunch cocktails. Our drinks menu is extensive with bottomless cocktails including the classic Bellini with peach puree, Mimosa with OJ and French 75s.*

Such actions in relation to the PHAA 2018 bring attention to the crucial issue of the enforcement of legislation in Ireland. Alcohol Action Ireland reports that they have repeatedly raised this issue with the HSE without any result [13]. Recent research has highlighted other deficits in the enforcement of the PHAA 2018 [14]. This lack of enforcement is also evident in other public health-related domains, including tobacco control [15], road traffic laws [16–19], and environmental protections [20]. It has been suggested that alcohol legislation enforcement in Ireland may be impeded by issues as legal complexities and apprehension relating to judicial enforcement [21]. The result may be an orientation towards soft mandates rather than prosecution.

This examination suggests that alcohol control legislation without robust enforcement may be of little use in restricting illegal alcohol promotion. Alcohol remains a commercial determinant of health that continues to significantly negatively impact population health in Ireland [6,7]. It is disconcerting that despite prior reports to the HSE on this issue [12], no action appears to have been taken. Ireland has exhibited a lack of leadership in relation to alcohol control in the past [22]. However, direct and purposeful leadership is now required to promote health and combat the threat posed by practices that actively promote excess alcohol consumption.

CONFLICT OF INTEREST

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The Importance of Hierarchical Regression in Public Health Data Modeling

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Keywords: Hierarchical Regression, Public Health, Outcome Prediction

Hierarchical regression is an important statistical technique through which multiple predictors can be tested for their impact on an outcome variable in an orderly, structured manner [1]. For public health, with complex, multi-level data, hierarchical regression is an important tool for determining the most impactful drivers of health outcomes and informing the development of useful interventions [2]. One of the main strengths of hierarchical regression over other regression techniques is its capacity for modeling the nested nature of the data. Public health data is typically collected at various hierarchical levels of analyses, such as individual, family, community, or geographic areas. Hierarchical regression enables the researcher to control for the interdependency within these different hierarchical levels as well as model individual-level measures and group-level measures influencing health outcomes [3].

Additionally, hierarchical regression allows researchers to test the independent contribution of every predictor variable toward the outcome variable with other variables held constant. This is especially useful in public health studies, as several variables may impact health outcomes at the same time. Using hierarchical regression, the researcher can identify what the strongest predictors of health care and target them accordingly for intervention [2, 4]. Furthermore, hierarchical regression offers insight into complex interactions between multiple predictor variables [5]. Public health issues tend to be complex in nature as they have an array of individual, social, economic, as well as environmental causes [6]. This method allows researchers to examine how different variables interact and better understand their roles in shaping health outcomes.

Additionally, hierarchical regression allows for the detection of potential modifiers or mediators of the relation between predictor variables and the outcome variable [7]. This is critical in public health research, as knowledge of the mechanisms by which the variables affect health is important in creating tailored interventions as well as policies. One of the key features of hierarchical regression in public health modeling is its capacity for measuring the influence of time-varying variables on health outcomes [8, 9]. Public health concerns are dynamic in nature and can evolve over time as a function of diverse sets of drivers, including policy shifts, social trends, or environmental phenomena [10]. Hierarchical regression enables researchers to identify such trends over time and include them in their models.

In addition, hierarchical regression allows researchers to control for confounding variables that could distort the association between health outcomes and predictor variables [11]. Public health data is often subject to confounding bias, where the association between variables is confounded by the effects of other variables. Hierarchical regression allows researchers to account for these confounding factors and obtain more accurate estimates of the true relationships between variables [12, 13]. Ultimately, hierarchical regression provides a flexible framework for evaluating whether observed relationships hold across subgroups or contexts. This makes it especially valuable in public health research, where interventions must often account for population-level variability and complex implementation environments.

By way of summary, hierarchical regression is an effective tool for public health data modeling that enables scientists to unravel the complexity of health determinants, discern the main drivers of health outcomes, and inform the establishment of effective interventions. Hierarchical regression can capture nested structures of the data, test interactions between variables, control for confounding variables, and test the generalizability of results, which makes the tool invaluable for public health investigation.

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AUTHORS CONTRIBUTIONS

All authors contributed to the conception, writing, and revision of this editorial. They read and approved the final version of the manuscript.

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More Days, Greater Health: Associations Between Daily MVPA and Cardiometabolic Risk in Youth

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SUMMARY

Objectives: To examine the associations of moderate-to-vigorous intensity physical activity (MVPA) with cardiometabolic health risks in children and adolescents. We also investigated the differences in MVPA between boys and girls and how the differences in MVPA affect cardiometabolic health.
Methods: Pooled data from 20 studies involving children and adolescents aged 3-18 years old from the International Children's Accelerometry Database (ICAD). MVPA was measured objectively by accelerometry. A Clustered Cardio-Metabolic Risk Score (CCMR) was calculated based on central adiposity, blood pressure, lipid profile, and glucose metabolism indicators.
Results: Boys are more active than girls per week in the study. In regression analysis, the MVPA-adjusted models for the fasting blood sample group indicated that CCMR in category 6 was significantly lower than in category 3($t = 2.41$, $p < .05$). The results are similar in the non-fasting blood sample group.
Conclusions: MVPA is associated with cardiometabolic health. More MVPA is beneficial for cardiometabolic health in children and adolescents.

Keywords: moderate-to-vigorous physical activity; cardiometabolic health.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death globally[1]. The CVD death burden increased significantly with ageing, and older people suffer from higher CVD deaths in Western Europe. More than 70% of CVD deaths are attributed to metabolic disorders[2]. Even though the prevalence of CVD is still higher in men, mortality associated with myocardial infarction is increasing in women, and a low socioeconomic context increases the global burden of CVD in women[3]. Finally, people living with low socioeconomic status exhibit more increased CVD event rates and poorer outcomes[4].

CVD risk factors include total and central adiposity, insulin resistance, blood lipids and lipoproteins, blood pressure, inflammatory proteins, and cardiorespiratory fitness. CVD is associated with obesity, diabetes, dyslipidemia, and hypertension[5], [6]. The relation between obesity, especially visceral obesity, and CVD appears to develop at a relatively young age[7]. The relationship between central obesity and CVD is complex. Some researchers have reported that the connection is indirect and dependent on an increased prevalence of diabetes, hypertension, and dyslipidemia. In contrast, others have found that obesity is an independent risk factor for CVD[8], [9], [10].

Regular physical activity (PA) is beneficial for health. Research demonstrated that meeting and maintaining the recommended moderate-to-vigorous intensity physical activity (MVPA) level could reduce cardiovascular risk for adults[11]. However, there is strong evidence that regular engagement in MVPA among children and adolescents is associated with significant health benefits[12]. Accordingly, the 2020 World Health Organization

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(WHO) guidelines on physical activity recommend that young people in this age group engage in at least 60 minutes of MVPA on average each day throughout the week, in addition to participating in vigorous-intensity physical activities and muscle- and bone-strengthening activities at least three days a week.

The 2020 WHO guidelines for children and adolescents introduced a notable shift, no longer requiring 60 minutes of MVPA every single day[13]. Instead, the guidelines allow for achieving "an average of 60 minutes per day" across the week. While the guidelines still encourage practicing MVPA daily, they now account for day-to-day variability.

This shift reflects the scientific evidence from studies that reported the average daily minutes of MVPA rather than an accumulation of 60 or more minutes[13], [14]. Thus, the new guidelines do not imply that there are no additional benefits from participating in MVPA every day but rather recognize the limitations of the existing evidence.

Measurements of PA include self-report methods such as questionnaires, activity logs, and diaries, as well as objective measurements of PA such as direct observation, doubly labelled water, heart rate monitoring, accelerometers, and pedometers[15]. Accelerometry in children and adults is the most commonly used objective PA measurement[15], [16]. Because accelerometers are small, they have a relatively low participant burden and cost. In addition, objective measures do not rely on information provided by the patient, but instead, measure and record the biomechanical or physiological consequences of performing PA, often in real time. As such, objective measures are less subject to the reporting bias or recall problems associated with self-report methods.

In summary, there is a knowledge gap regarding how many days of MVPA are necessary to make a significant difference in the health benefits for young people. Therefore, this study aims to investigate the associations of MVPA with cardiometabolic health risks in children and adolescents using the International Children's Accelerometry Database (ICAD).

METHODS

Participants

The ICAD is a pooled dataset comprising data from 20 studies involving children and adolescents aged 3 to 18 years old, who had their physical activity levels measured via ActiGraph accelerometer (ActiGraph, LLC, Pensacola, FL, USA). For the current analysis, only studies that collected blood samples and required participants to wear an ActiGraph device for seven consecutive days were included. The final sample comprised 5,284 participants from two studies conducted in the United States. After excluding cases with missing values for cardiometabolic risk variables (n=1,927) and Actigraph daily wear-time (n=51), the final sample included 3,306 participants. Among these, 1,308 had fasting blood samples, while 1,998 did not.

We obtained the ICAD data use agreement to present it in the paper.

Measures

Moderate- to vigorous-intensity physical activity (MVPA): Actigraph raw data were summarized into day-level variables, including wear-time and PA. An epoch length of 60 seconds, a minimum wear-time of 8 hours per day, and a threshold of 2000 intensity counts per minute (CPM) for MVPA were applied. Any day-level MVPA minutes with wear-time shorter than 8 hours were treated as missing values and subsequently imputations using multiple imputations with chained equations (MICE) in Stata 17.0. Twenty complete datasets were created and used for pooled estimation when analyses involved the imputed values. After testing associations with the missing pattern, MICE incorporated ethnicity, type of blood sample (fasting or non-fasting), and the proportion of MVPA per wear-time when wear-time was shorter than 8 hours as auxiliary variables. Day-level MVPA minutes were aggregated to compute total MVPA minutes per week and determine MVPA adherence. MVPA adherence was categorized into five dummy-coded groups: category 0 = did not meet the WHO MVPA guideline (<420 minutes of MVPA per week), and categories 3 through 6 = met the WHO MVPA guideline, with the number of active days (defined as days with 60 or more minutes of MVPA) specified as follows: 1 to 3 days (category 3), 4 days (category 4), 5 days (category 5), and 6 to 7 days (category 6). To balance cell sizes, participants with 1 to 3 active days were combined into category 3, and those with 6 to 7 active days were merged into category 6. Categories are as follows:

Clustered Cardio-Metabolic Risk Score (CCMR): A CCMR was calculated based on central adiposity,

Table 1. MVPA adherence categories

Category	definition	days
Category 0	did not meet the WHO MVPA guideline (<420 minutes of MVPA per week)	0
Category 3	met the WHO MVPA guideline, with the number of active days (defined as days with 60 or more minutes of MVPA)	1 to 3
Category 4		4
Category 5		5
Category 6		6 to 7

blood pressure, lipid profile, and glucose metabolism indicators. These indicators were standardized as sex-specific z-scores. Triglycerides and insulin, which were highly skewed, were normalized using natural log transformation before standardization. Systolic and diastolic blood pressure were averaged. For participants with fasting blood samples, CCMR was calculated as the sum of standardized waist circumference, average of systolic and diastolic blood pressure, triglycerides, glucose, and insulin, subtracted by HDL-cholesterol. CCMR for non-fasting blood samples was calculated without triglycerides, glucose, and insulin, as these indicators are sensitive to food intake before the examination.

Statistical Analysis

Descriptive statistics, including means and standard deviations for age, cardio-metabolic risk indicators, and CCMRs, were calculated for the overall sample, as well as for boys, and girls separately. Estimated mean values with 95% confidence intervals were presented for MVPA minutes per week and MVPA adherence due to the use of imputed data.

Age-adjusted linear regression models were tested using twenty complete datasets from multiple imputations, with MVPA adherence as the independent variables and fasting CCMR or non-fasting CCMR as the dependent variables separately, stratified by sex [overall (age- and sex-adjusted), boys, girls]. Each model was run with and without adjustment for weekly MVPA minutes to explore associations between CCMR and additional active days while holding weekly MVPA constant. When MVPA adherence was statistically significant, Wald tests were performed post-hoc to compare the strength of associations across adherence categories. All analyses were conducted in Stata 17.0, with significance set at $p<0.05$.

RESULTS

Characteristics of all participants

Descriptive statistics stratified by sex are presented in Table 2. The average MVPA minutes per week was 331 minutes. Boys and girls engaged in 396 and 264 minutes of MVPA per week, respectively. The adherence rate to the 2020 WHO MVPA guideline (i.e. an average of 60 or more minutes of MVPA per day per week = 420 or more minutes per week) was 27.2% (95% CI = 25.6–28.7%). Sex-specific adherence rates were 38.9% (95% CI = 36.5–41.4%) for boys and 15.0% (95% CI = 13.2–16.8%) for girls.

Associations between moderate-to-vigorous physical activity adherence and cardiometabolic risk in children and adolescents

Results from MVPA-adjusted regression models for boys and girls separately, as well as MVPA non-adjusted models, are presented in Table 3.

The MVPA-adjusted models for the fasting blood sample group indicated that CCMR was significantly lower in categories 3 (B = -0.989, 95% CI = –1.945 to –0.034), 4 (B = –1.852, 95% CI = –2.753 to –0.952), 5 (B = –1.856, 95% CI = –2.800 to –0.911), and 6 (B = –2.531, 95% CI = –3.728 to –1.335) compared to category 0, controlling for age and sex. Post-hoc analyses revealed that CCMR in category 6 was significantly lower than in category 3 ($t = 2.41$, $p < 0.05$).

For the non-fasting blood sample group, the MVPA-adjusted models showed that CCMR was significantly lower in categories 4 (B = –0.454, 95% CI = –0.838 to –0.069), 5 (B = –0.845, 95% CI = –1.238 to –0.453), and 6 (B = –0.676, 95% CI = –1.183 to –0.170) compared to category 0. However, category 3 (B = –0.010, 95% CI = –0.422 to –0.402) did not differ significantly from category 0. Wald tests indicated that categories 5 ($t = 3.61$, $p < .001$) and 6 ($t = 2.59$, $p < 0.05$) had significantly lower CCMR compared to category 3.

DISCUSSION

The present study examined the association of MVPA with cardiometabolic health risk in children and adolescents using the ICAD database including studies using objective measurement of PA via actigraphy. The main of the study results are: 1) the number of active days, independent of accumulating an average of 60 or more minutes of daily MVPA (i.e. 420 or more minutes of weekly MVPA), was significantly associated with reductions in cardiometabolic risk in children and adolescents; 2) boys had a higher adherence rate of MVPA and were more active than girls which is consistent with other previous research[17], [18], [19].

Obesity in youth is one of the major health concerns worldwide, notably because of the incidence of poor cardiovascular health in this population. Indeed, it has been demonstrated in children aged from 2 to 15 years old that nearly 20% exhibit fibrous-plaque lesions in the aorta and that 8% have coronary vessel lesions[20]. In addition, it has been demonstrated that adolescents have a high prevalence of advanced atherosclerotic coronary artery plaques[21]. Because of the early altered cardiovascular health, children and adolescents are at risk of having cardiovascular events during their adulthood[22]. In this context, it is important to propose new strategies to prevent obesity in youth and its associated comorbidity and mortality.

Regular physical activity could represent one of the strategies because of its beneficial effects on children with obesity. It has been demonstrated that exercise training is able to improve pulse wave velocity and carotid intima-media thickness markers in children[23]. Moreover, regular physical activity appears to be able

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Table 2. Descriptive characteristics of all participants and stratified by sex

Variables	Total (n=3,306)	Boys (n=1,677)	Girls (n=1,629)
	Mean (SD)	Mean (SD)	Mean (SD)
Age (years)	13.97 (2.81)	14.04 (2.76)	13.89 (2.86)
Fasting plasma glucose (mmol/L)	5.13 (0.81)	5.24 (0.9)	5.01 (0.69)
Fasting serum insulin (pmol/L)	90.81 (81.64)	87.92 (87.33)	94.04 (74.69)
HDL-cholesterol (mmol/L)	1.39 (0.33)	1.36 (0.33)	1.42 (0.33)
Fasting plasma triglycerides (mmol/L)	0.98 (0.68)	0.98 (0.78)	0.97 (0.56)
Systolic blood pressure (mmHg)	108.06 (10.41)	109.93 (10.7)	106.14 (9.72)
Diastolic blood pressure (mmHg)	57.37 (12.31)	56.54 (13.07)	58.23 (11.41)
Waist circumference (cm)	78.08 (15)	77.75 (15.53)	78.42 (14.43)
CCMR fasting samples	0.620 (3.481)	0.614 (3.556)	0.627 (3.399)
CCMR non-fasting samples	0.003 (2.089)	0.031 (2.182)	–0.025 (1.993)
MVPA (minutes/week)	330.72 (4.61)	395.87 (6.48)	263.65 (6.04)
MVPA adherence (%)[1]			
Not adhered (%)	72.85 (71.26 to 74.44)	61.06 (58.61 to 63.52)	84.98 (83.16 to 86.79)
Adhered (%)	27.15 (25.56 to 28.74)	38.94 (36.48 to 41.39)	15.02 (13.20 to 16.84)
Adhered and for 1 day (%)[2]	0.62 (0.3 to 0.93)	0.47 (0.03 to 0.9)	0.77 (0.27 to 1.27)
Adhered and active for 2 days (%)	1.80 (1.27 to 2.34)	2.20 (1.38 to 3.02)	1.39 (0.71 to 2.07)
Adhered and active for 3 days (%)	4.29 (3.51 to 5.07)	5.98 (4.67 to 7.3)	2.54 (1.68 to 3.4)
Adhered and active for 4 days (%)	6.96 (6.03 to 7.9)	10.16 (8.6 to 11.72)	3.67 (2.71 to 4.62)
Adhered and active for 5 days (%)	7.49 (6.56 to 8.42)	11.05 (9.45 to 12.66)	3.82 (2.83 to 4.81)
Adhered and active for 6 days (%)	3.98 (3.28 to 4.67)	6.03 (4.85 to 7.22)	1.86 (1.13 to 2.59)
Adhered and active for 7 days (%)	2.02 (1.53 to 2.51)	3.03 (2.2 to 3.86)	0.98 (0.49 to 1.47)

Abbreviations: SD standard deviation, MVPA= moderate- to vigorous physical activity.

1) MVPA adherence = the proportion of individuals who participated in 420 minutes or more minutes of MVPA during a week. Proportions (%) and 95% confidence intervals in parentheses are presented instead of means and standard deviations.

2) The proportion of individuals who participated in 420 or more minutes of MVPA and the number of days with 60 or more minutes of MVPA was only one day.

Table 3. Associations between MVPA adherence and cardiometabolic risk in children and adolescents

Variables	All (n=3,306)[1]		Boys (n=1,677)[2]		Girls (n=1,629)[2]	
	B (95% CI)	Wald-test[3]	B (95% CI)	Wald-test	B (95% CI)	Wald-test
$CCMR_{fasting}$	n=1308		n=686		n=622	
MVPA min/week adjusted model						
420 mins/wk (C0)	Reference group					
<420 mins/wk, 1-3 activ<e days (C3)	–0.989 (–1.945 to –0.034)	C3>C6	–0.502 (–1.689 to 0.685)		–0.599 (–2.338 to1.141)	
<420 mins/wk, 4 active days (C4)	–1.852 (–2.753 to –0.952)		–1.057 (–2.254 to 0.139)		–1.477 (–3.524 to 0.57)	
<420 mins/wk, 5 active days (C5)	–1.856 (–2.8 to –0.911)		–1.024 (–2.265 to 0.217)		–1.184 (–3.115 to 0.747)	
<420 mins/wk, 6–7 active days (C6)	–2.531 (–3.728 to –1.335)		–1.396 (–3.023 to 0.232)		–1.247 (–3.769 to 1.274)	
MVPA min/week unadjusted model						
<420 mins/wk (C0)	Reference group					
<420 mins/wk, 1-3 active days (C3)	–0.793 (–1.649 to 0.063)	C3>C6	–1.092 (–2.102 to –0.082)		–0.16 (–1.778 to1.459)	
<420 mins/wk, 4 active days (C4)	–1.652 (–2.466 to –0.837)		–1.735 (–2.638 to –0.832)	C3>C6	–1.062 (–3.08 to 0.957)	
<420 mins/wk, 5 active days (C5)	–1.635 (–2.486 to –0.784)		–1.794 (–2.736 to –0.853)		–0.795 (–2.665 to 1.075)	
<420 mins/wk, 6-7 active days (C6)	–2.145 (–3.097 to –1.192)		–2.527 (–3.602 to –1.452)		–0.105 (–2.161 to 1.95)	
$CCMR_{non-fasting}$	n=1998		n=991		n=1007	
MVPA min/week adjusted model						
<420 mins/wk (C0)	Reference group					
<420 mins/wk, 1-3 active days (C3)	–0.01 (–0.422 to 0.402)	C3>C5, C6	–0.236 (–0.776 to 0.305)	C3>C5, C6 C4>C5	0.472 (–0.239 to 1.184)	
<420 mins/wk, 4 active days (C4)	–0.454 (–0.838 to –0.069)		–0.473 (–0.946 to 0.000)		–0.282 (–0.955 to 0.392)	
<420 mins/wk, 5 active days (C5)	–0.845 (–1.238 to –0.453)		–1.103 (–1.576 to –0.63)		–0.181 (–0.871 to 0.509)	
<420 mins/wk, 6-7 active days (C6)	–0.676 (–1.183 to –0.17)		–0.949 (–1.537 to –0.362)		0.361 (–0.557 to 1.28)	
MVPA min/week unadjusted model						
<420 mins/wk (C0)	Reference group					
<420 mins/wk, 1-3 active days (C3)	–0.071 (–0.432 to 0.29)	C3>C5, C6	–0.186 (–0.662 to 0.291)	C3>C5, C6 C4>C5	0.003 (–0.599 to 0.605)	
<420 mins/wk, 4 active days (C4)	–0.515 (–0.856 to –0.174)		–0.419 (–0.844 to 0.006)		–0.729 (–1.306 to –0.152)	
<420 mins/wk, 5 active days (C5)	–0.919 (–1.237 to –0.6)		–1.036 (–1.417 to –0.654)		–0.67 (–1.257 to –0.084)	
<420 mins/wk, 6-7 active days (C6)	–0.789 (–1.137 to –0.441)		–0.847 (–1.249 to –0.444)		–0.392 (–1.058 to 0.274)	

Abbreviations: $CCMR_{fasting}$ = Clustered Cardio-Metabolic Risk score for individuals with fasting blood samples (calculated by summing the standardized serum glucose, insulin, triglycerides, blood pressure, and waist circumference, then subtracting the serum HDL-cholesterol level), $CCMR_{non-fasting}$ = Clustered Cardio-Metabolic Risk score for individuals with non-fasting blood samples (calculated by summing the standardized blood pressure and waist circumference, then subtracting the serum HDL-cholesterol level).

1) age- and sex-adjusted models.

2) age-adjusted models.

3) Post-hoc Wald-test tested differences between the regression coefficient among the MVPA adherence categories at alpha=0.05 (*e.g.* C3>C5, C6 indicates that the regression coefficients of C5 and C6 are significantly greater than C3).

to improve vascular function and induce beneficial changes in fat and lean body mass in children and adolescents with obesity[24].

Current WHO guidelines, along with most national physical activity recommendations, specify a minimum volume of MVPA (a combination of intensity, duration, and frequency) for children and adolescents[25]. These guidelines also state that exceeding the minimum recommended volume may offer additional health benefits, such as adiposity reduction. However, evidence supporting the dose-response relationship between daily MVPA volume and health benefits for this age group has been limited[26].

Moreover, evidence linking the frequency of MVPA and health risk reduction has not yet been well established. According to Tremblay et al.[14], The most supporting evidence for 60 minutes of daily MVPA was based upon "average" daily MVPA, which is calculated as the total MVPA divided by the measurement periods. Acknowledging this limitation, the 2020 WHO guidelines now recommend that children and adolescents aim for an "average" of 60 minutes per day of MVPA [12].

In this context, the investigation of the relationship between the number of days children and adolescents engaged in 60 or more minutes of MVPA and cardiometabolic risk revealed that performing 60 or more minutes of MVPA on as many days as possible throughout the week was correlated with a lower CCMR. Thus, engaging in MVPA consistently, rather than accumulating activity over fewer days, appears to be more effective in reducing cardiometabolic risk. It should be interesting for young people to have consistent PA because it has been shown that consistency in PA is related to greater MVPA and potential exercise routine stability that may induce greater health benefits [27]. The consistency of PA may also limit or prevent the risk of becoming obese and suffering from its complications during adulthood. The consistency of PA should also be beneficial, especially for girls who should be mothers one day in the early prevention of non-communicable diseases for their future children[28].

Acute and long-term improvements in metabolic function and cardiovascular fitness resulting from regular MVPA provide a plausible explanation for these points. Research suggests that PA may have favorable effects among patients with insulin resistance, metabolic syndrome, type 2 diabetes or obesity[29], [30]. Regular exercise provides many benefits, like improvements in blood glucose control and the ability to prevent or delay type 2 diabetes. PA improves lipid metabolism and blood pressure; it may also reduce total daily insulin requirements in people on insulin treatment and is at least as effective in diabetes prevention as medicines[30], [31].

This study, however, has limitations. First, it relied on cross-sectional data, which restricts the ability to establish a cause-and-effect relationship between MVPA and cardiometabolic health risks. Second, the analyses did not account for other known behavioral risk factors, such as nutritional intake. While the aim of this paper was to explore how effective MVPA is in mitigating risks associated with cardiovascular diseases in children and adolescents, the condition is not caused by just the conditions reported as measures in the selected sources of evidence or themes in the findings section. As such, a correlation between MVPA and a reduction in the risks of cardiovascular diseases cannot be verified. Further studies are thus needed to include more cardiovascular risk factors and empirically investigate how they are affected by MVPA.

Possible proposals and solutions provided

- To convince parents, schools, communities, and governments that children should be provided with opportunities to engage in moderate-to-vigorous physical activity (MVPA) on as many days as possible throughout the week, not only during physical education classes but also through extracurricular activities.
- To educate children and their parents about the importance of regular physical activity in improving cardiometabolic health and reducing the risk of cardiovascular disease (CVD) in adulthood.
- To encourage researchers to investigate the additional benefits of frequent MVPA, independent of total volume, through longitudinal studies.

CONCLUSION

In conclusion, physical activity, especially consistent MVPA, is important for cardiometabolic health in children and adolescents. To prevent the clustering of cardiovascular disease risk factors, further research should focus on the duration and amount of MVPA, the mechanism of cardiovascular disease and their associations in this specific population.

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Distributed Lag Non-Linear Models for the impact of heat waves on elderly people living in the regions of central-eastern Italy

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SUMMARY

Background: Prolonged periods of extreme heat, usually referred to as heat waves, have a significant impact on health, especially in the most vulnerable populations. In the present study, we investigated the effect of heat waves on mortality in the elderly population living in the regions of central-eastern Italy.
Methods: We considered 10 cities located between the Marche and Abruzzo regions during the period 2011–2021. The association between heat waves and mortality risk was analysed for each city using non-linear distributed lagged temperature and humidity functions, a method that accounts for non-linear lagged effects, including the harvest effect, a phenomenon where mortality decreases temporarily after an initial peak due to early deaths of vulnerable individuals. We then performed a multivariate meta-analysis on all cities to jointly synthesise multiple results on mortality risk during heat waves, taking into account their correlation.
Results: In the first days after the heat wave, the relative risk (RR) tends to increase, then decreases with a lag of about 4 days and then stabilises around the reference value (RR = 1), with a slight increase around day 21–22.
Conclusion: The study shows a significant increase in risk in the presence or after the occurrence of a heat wave. The heterogeneous behaviour of some cities could be due to other factors (e.g. pollution) that need to be investigated. The aggregate analysis allows a more robust estimate of the overall effect, reducing the uncertainty arising from individual local analyses.

Keywords: Distributed Lag Nonlinear Models, Multivariate Meta-Analysis, Heat Waves, Multivariate Time Series

INTRODUCTION

It is important to analyse the health impact of extreme heat and heat waves, especially on the elderly population, as their frequency and intensity are expected to increase under projected climate change scenarios.

Many epidemiological studies have documented that prolonged periods of extreme heat, i.e. the heat waves, are associated with a significant increase in mortality.

Sometimes the effects of prolonged exposure to extreme temperatures are not limited to the period in which they are observed but are delayed in time.

The short-term effects of exposure to high levels of extreme temperatures affect health within a few days of the event. This is known as the "harvesting effect": heat waves hit the most vulnerable people after a short period of time. After the initial effect, the number of cases decreases a few days later.

In [1] the authors analyzed the mortality risk during heat waves in several USA cities through generalized linear models, combining individual estimates of the effect of heat waves with hierarchical Bayesian models.

Gasparrini et at. introduced the Distributed Lag Non-Linear Models (DLNM) to describe the complex relationship between extreme heat and heat waves and mortality [2–5].

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DLNM models describe the exposure-response relationship to extreme heat or an intense pollution event through a sequence of possible future scenarios. The associated risk was estimated using a non-linear distributed lag temperature function, which accounts for non-linear lag effects and term harvesting.

In [6], the authors used time-series regression analysis to estimate site-specific temperature-mortality associations and then performed a meta-analysis of multiple geographical locations.

Alicandro et al. in [7] analysed the excess of mortality due the Omicron variant in Italy during April-July 2022, in particular at working age, by using over-dispersed Poisson regression models.

In this study, we considered 10 cities located between Marche and Abruzzo in the period 2011–2021, using distributed nonlinear lag functions of temperature and humidity, which take into account nonlinear lag effects. We first analysed the impact of extreme temperatures on health, considering each day as independent. In particular, we took as reference temperatures the 95th and 99.9th percentiles of annual temperatures, which vary depending on the city considered, and studied the risk trend in the days immediately following the occurrence of the extreme temperature. We then estimated the effect of several consecutive days of extreme heat (heat wave) on mortality among the elderly, depending on its duration and time lag. In the presence of heat waves, the risk increases in intensity and duration compared to the previous case where the temperature was set regardless of whether extreme temperatures could continue in the following days. There are similarities and differences in how different cities respond to heat waves. After analysing each city using the DLNM technique, we performed a multivariate meta-analysis across all cities to summarise the multiple findings on mortality risk during heat waves, taking into account their correlation.

DATA AND METHODS

Data

As case studies, we considered 10 cities located between the Marche and Abruzzo regions: Alba Adriatica, Ancona, Pescara, San Benedetto del Tronto, Giulianova, Martinsicuro, Pineto, Roseto, Silvi, Tortoreto, in the period 2011–2021. The series of daily all-cause mortality data consist of the number of deaths among the inhabitants of each town and were extracted from ISTAT.

For the weather data, we obtained hourly measurements of temperature, humidity and other variables from worldweather.wmo.int. This data is divided into 4 groups, each of which includes some nearby cities. The first group includes Ancona, the second San Benedetto del Tronto and Martinsicuro, the third Alba Adriatica, Tortoreto, Giulianova and Roseto, the fourth Pineto, Silvi and Pescara. The maximum temperature is calculated as the highest hourly value recorded for each day, while the average temperature and humidity are calculated as the average of the hourly values recorded for each day.

Statistical analysis

The same common DLNM model was applied to each community and the studies were then aggregated using meta-analysis.

DLNM models were implemented, which allow us to describe in detail both the non-linear nature of the association between temperature and mortality, and the lag with which the effects manifest themselves.

We consider the following formula:

$$log\left(E\left(Y_t\right)\right)=\alpha+\sum_{j=1}^{v_x}\sum_{k=1}^{v_l} r^T\left(t\right)_{j.}\, c_{.k}\beta_{jk} \qquad (1)$$

where the process Y_t measures the number of deaths, assumed to follow an overdispersed Poisson distribution for each day $t, 1\le t\le n$. The symbol $E\left(Y_t\right)$ denotes the expected number of deaths on day t. The vector $\vec{x}$ is the $N-$dimensional exposure series, in particular, we first consider the maximum temperature on day t as x_t. The vector $\vec{l}$ is the lag: $\vec{l}=(0,1,\ldots,L).C$ is the $(L+1)\times v_l$ matrix of the basis variable derived by applying the specific basis function to the lag vector $\vec{l}$. The $n\times v_x\times(L+1)$ matrix element $r^T\left(t\right)_{j.}$ represents the basis variable for the lagged exposure at time t. The parameters β_{jk} are to be estimated.

Then we take the heat wave as the exposure variable $\vec{x}$. The heat wave variable is a binary indicator: it takes the value 1 if a heat wave occurs, otherwise it takes the value 0.

RESULTS

To assess the effect of temperature on mortality in the over-75s, R programs were developed, based on data from the different cities analysed, starting with the package dlnm [2] created by Gasparrini et al. The analysis considers both immediate and delayed effects, up to a maximum of 30 days.

We first analysed model (1) using a cross base with a natural cubic spline with 5 degrees of freedom, which captures the non-linear lagged effects over 30 days and the effects of temperature (maximum daily temperature) above a threshold of 30°. We also include as confounders the day of the week as a categorical variable to account for weekly seasonality, the day of year, in order to describe the seasonal effect within each year, and mean daily humidity as an instantaneous, not lagged variable.

The relative risk (RR) depends on both the temperature and the delay. Fig. 1 illustrates how the temperature-related risk evolves over time in Ancona. In

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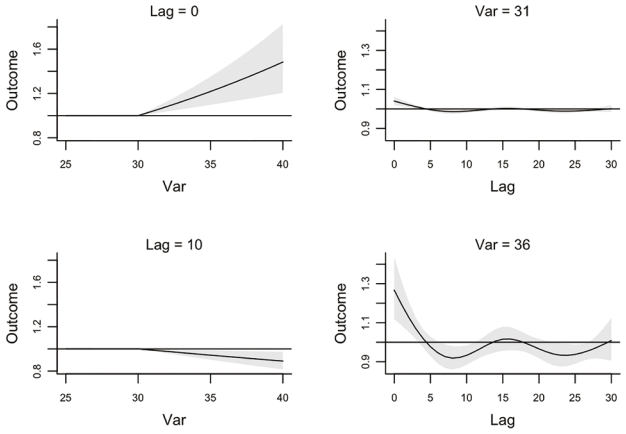
the top left graph, where the lag is zero, the immediate effect of temperature on mortality is observed: the risk increases with temperature, following an exponential trend. For temperatures above 30°C, the increase in risk is almost linear, while the statistical uncertainty (grey area) increases significantly above 35°C. In the lower left graph, the risk flattens out with a lag of 10 days, indicating that temperature no longer has a significant effect on mortality after this interval. The graphs on the right show the evolution of the risk by fixing certain temperatures. At a temperature of 31°C (95th percentile, top right graph), the initial risk is slightly above 1 ($\sim 1.040, CI = (1.019; 1.062)$), and remains above 1 for about four days before stabilising around 1 with longer lags. The narrow confidence interval indicates a reliable estimate. At 36°C (99.9th percentile, bottom right graph) the initial risk is significantly higher, around 1.267 ($CI = (1.118; 1.435)$), and remains above 1 for about four days. It then decreases, but after about 15–16 days, a slight increase in risk is observed. For longer lags, uncertainty increases and moderate oscillations are observed. These results suggest that very high temperatures have an immediate effect on mortality.

Figure 1. Plot of RR by temperature in Ancona at specific lags (left), RR by lag at 95th and 99.9th percentiles of temperature distribution (right)



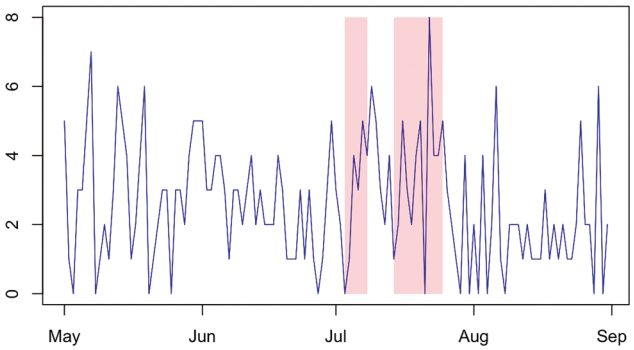
Heat waves

After analysing the impact of daily temperatures considered independently, other codes were developed in R to study the impact of heat waves. As the available data is limited to the last 10 years, during which there has been a significant increase in the mean temperature, we have adopted the following definition of a heatwave: a period of at least three consecutive days on which the maximum daily temperature exceeds the baseline. The baseline is defined as the sum of the historical daily mean temperature and historical daily standard deviation for each day between 2011 and 2021.

The aim is to estimate for how many days the effect of the heat wave on mortality persists in relation to its duration. Compared to the previous case, in which a temperature was fixed independently of the consecutive days in which it lasted, the risk increases in intensity and duration. The inclusion of the lag term allows us to examine how the effect of a heat wave evolves over time, identifying both an immediate increase in risk and possible delayed effects, such as the harvesting effect.

The graph in Figure 2 shows the evolution of deaths among people over 75 in Ancona in the period May-September 2015, highlighting the days characterised by heat waves with red rectangles. It can be observed that heat waves are mainly concentrated in the central summer months, between June and August, with variable duration. However, not all waves are associated with a clear increase in mortality, suggesting that factors such as the intensity of the event and the population's ability to adapt may play a decisive role. We chose to focus on Ancona in 2015 because it experienced the longest heat wave of the 2011–2021 period, lasting 12 days. In particular, one of the highest mortality peaks of the entire period was recorded immediately after the first wave in July. Furthermore, the absolute maximum of eight deaths occurred within the second, longer wave, suggesting a possible cumulative effect between the previous and the current wave. Finally, a comparison of the pre- and post-wave periods shows greater stability before the event, while mortality peaks become more irregular afterwards, confirming the impact of heat waves on the elderly population.

Figure 2. Deaths of over-75s during the hot months of 2015 in Ancona, red zones indicate heat waves



The graph in Figure 3 provides a rough idea of how the duration of the heat wave affects the risk of death, as shown in the graph. To construct it, the baseline mortality on days without heat waves was estimated by calculating the average daily number of deaths among people aged 75 and over during these periods. This average is used as a reference to assess the increase in risk on days with heat waves. For each heat wave, the average mortality within the corresponding time window was then calculated. The relative risk (RR) was obtained as the ratio of the average mortality during the heatwave to the baseline mortality:

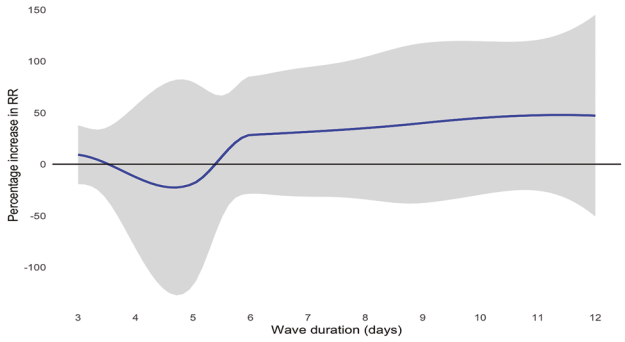
$$RR = \frac{Average\ mortality\ during\ the\ heatwave}{Baseline\ mortality}$$

From here, the percentage increase in risk was calculated as:

$$Percentage\,increase = (RR - 1) \cdot 100$$

The resulting graph uses a smooth curve to highlight the general trend. Initially, the risk is positive but moderate, then it decreases until it is around 4–5 days. However, during this period there is a widening of the confidence interval, indicating greater uncertainty in the estimates due to the lack of data for waves of this duration. After this point, the risk shows a clear exponential increase as the duration of the wave increases, confirming that the longest waves are particularly dangerous for the mortality of the elderly.

Figure 3. Percentage increase in risk as a function of wave duration in Ancona



To investigate the effect of heat waves on the mortality of people over 75 years of age, we considered model (1), which incorporates a quasi-Poisson family with non-linear effects of the lag function. We chose a natural cubic spline with five degrees of freedom and a linear exposure-response relationship. We also included the day of the week, the day of the year and mean daily humidity as confounders. The heatwave variable was included as a binary indicator (1 if a heatwave occurred, 0 if it did not).

In Ancona (Figure 4), the effect of heat waves on mortality lasts about 6 days. The initial risk is high (about 1.071, CI(0.991;1.157)) but gradually decreases over time. The reliability of the estimates is confirmed by the relatively narrow confidence intervals, indicating a robust model. After the seventh day, the risk is no longer statistically significant, suggesting that the impact of heat waves in this city is intense but short-lived.

In Pescara (Figure 5), the effect of heat waves on mortality also lasts for about 4 days. The initial relative risk is slightly lower than in Ancona (~ 1.019, CI(0.990;1.050)) and gradually decreases over time. The confidence intervals are similar to those observed in Ancona and still provide a reasonable level of confidence in the estimates. After the fourth day, the risk is no longer statistically significant, suggesting that the impact of heat waves in this city is milder but follows

Figure 4. Lag-response for heat wave days in Ancona

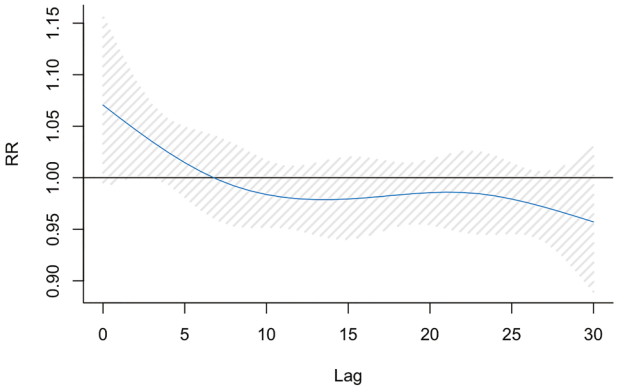
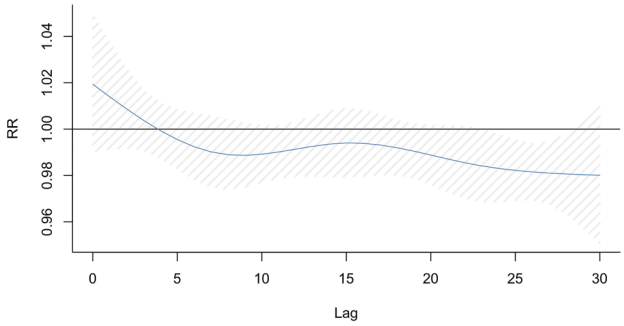


Figure 5. Lag-response for heat wave days in Pescara



a similar temporal pattern. These results underline that the characteristics of heat waves, their intensity - duration and local environmental conditions - play a crucial role in determining their impact on mortality. In addition, different populations react differently to them.

Sensitivity Analysis

To assess the robustness of the results to model specification, a sensitivity analysis was conducted by varying the degrees of freedom (df) used in the lag-response function of the distributed lag non-linear model (DLNM). The original model employed 5 degrees of freedom to capture the temporal structure of the lagged association. For the sensitivity analysis, alternative models were estimated using 3 and 6 degrees of freedom, representing lower and higher levels of spline flexibility, respectively. The goal was to evaluate whether the estimated exposure-lag-response relationship and effect estimates were influenced by the choice of df. The overall association patterns remained consistent across all three specifications. While minor variations were observed, particularly at longer lag periods, the magnitude and direction of effects did not materially change. These results suggest that the findings are robust to the specification of the lag spline, reinforcing the reliability of the main conclusions.

Regarding the heat wave model, we attempted to incorporate maximum temperature as a confounding factor. However, no significant changes were noted,

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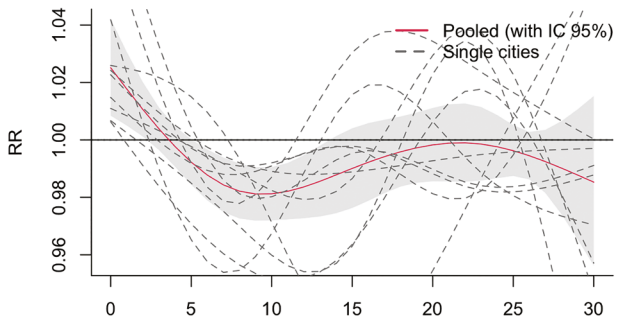
only a very slight increase in risk (with a percentage variation < 3%).

To clarify the fact that neighbouring cities have the same temperatures, a meta-analysis was conducted, considering only the main cities that had a weather station. The results obtained were not very different to those of the model including all cities.

Multivariate meta-analysis

Having analysed the impact of heatwaves in each city using DLNM models, we applied a meta-analytic approach to synthesise the results at an aggregate level. This method allows us to combine estimates from different locations while accounting for variability and potential correlations, thereby improving the precision and generalisability of the results. We used a multivariate approach to model several parameters simultaneously, taking into account the dependency structure between the cities analysed. A random effects model was used to deal with heterogeneity between cities and to produce more robust estimates. Specifically, we estimated the relative risk (RR) of mortality associated with heat waves with a lag of up to 30 days. For this analysis, we examined the same 10 cities that were analysed individually at the beginning. The graph in Figure 6 shows the trend in the RR for the population aged 75 and over as a function of lag. The dashed lines represent estimates for individual cities, while the red solid line shows the pooled RR, with the grey area showing the 95% confidence interval. At the begin the RR is about 1.025 ($CI = (1.008; 1.042)$). In the first days after a heat wave, the RR tends to decrease, remaining above 1 for about 4 days. It then stabilises under the reference value (RR = 1), with a slight increase around day 22: the most vulnerable population is affected first, followed by a temporary decline in mortality. This pattern suggests a possible delayed effect of heat waves, with an initial increase in risk followed by a period of compensation. The high variability between cities, highlighted by the dashed lines, reflects local differences in climate, demographics and health systems. However, the pooled analysis provides a more robust overall estimate, reducing the uncertainty from individual local analyses.

Figure 6. Effect of heat waves on mortality for over-75s



DISCUSSION

In the present study, we investigated the non-linear exposure-response relationship between temperature and mortality using multivariate time series data from ten cities in central-eastern Italy. The analysis was performed in three steps: in the first step, we examined the effect of extreme temperatures on mortality in people aged over 75 years, considering each day as independent. In particular, we used the 95th and 99.9th percentiles of annual temperatures as reference temperatures and observed the lagged effects of risk immediately after the day of extreme heat. In a second step, we considered heat waves, i.e. subsequent days in which an extreme temperature persists. In this case, risk is affected by the prolongation of hot days, and so is the lag. Although in some cases the weather data measurements are the same for neighbouring cities, the effect of the heat wave on the health of the elderly population could vary significantly because the time series counting the number of deaths could have a different distribution. Finally, we carried out a multivariate analysis to extrapolate from the characteristics of each city to the aggregate behaviour of the area in which the cities are located. Multivariate meta-analysis is a useful analytical tool [8] for studying complex associations between different cities and allowed us to obtain a behaviour that goes beyond the specificities of each city, providing an estimate of a lag of about 4–5 days for the area between Marche and Abruzzo. In this type of analysis, it is not necessary to interpret the parameters individually, since they are studied through their joint distribution. It could be interesting to include in the model variables of interest, such as the historical series of pollution rates, which would allow us to better understand, in addition to the weather data, the distribution of the delay after the heat wave.

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Blood donation in young people: estimation of education and awareness in Rome high school students using translated questionnaires

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SUMMARY

Background: Ensuring a stable blood supply is a critical public health challenge, with young adults representing a vital pool of potential donors. Education and awareness are key to fostering positive attitudes and behaviors toward blood donation among youth.
Objectives: This study aimed to assess the impact of educational interventions on the willingness of high school students in Rome to donate blood and to validate the Italian translation of two established questionnaires measuring knowledge and motivation related to blood donation.
Materials and methods: An observational, prospective, cross-sectional study was conducted among senior students at the "Democrito" high school in Rome during the 2022–23 academic year. Eligible students (aged 18 or older) completed two validated and translated questionnaires before and after a series of educational lectures on blood donation. The reliability and content validity of the translated instruments were evaluated, and changes in students' intentions and attitudes were analyzed using the McNemar test.
Results: Of 54 students with matched pre- and post-intervention responses, the proportion expressing a positive attitude toward blood donation increased from 57.4% to 75.9% following the educational sessions (p = 0.0213), indicating a statistically significant improvement. Motivations for donation included altruism, health benefits, social approval, and practical incentives. Despite increased willingness, only a small fraction of students who expressed intent ultimately donated blood, highlighting a gap between intention and action. The translated questionnaires demonstrated high content validity (CVI: 0.975 and 0.908) and were well-understood by the target population.
Conclusions: Educational interventions significantly enhanced students' willingness to donate blood, underscoring the importance of targeted awareness campaigns in schools. However, bridging the gap between intention and actual donation remains a challenge, necessitating further strategies to address logistical and psychological barriers. The validated Italian questionnaires provide reliable tools for future research and program evaluation in this context.

Keywords: blood donors; blood donation; delivery of healthcare; surveys and questionnaires; schools.

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INTRODUCTION

Blood donation is vital, as it saves lives. Regular blood donation by healthy individuals is necessary to ensure that blood is always available. Globally, around 118.54 million blood donations are collected annually [1]. Blood is an essential resource, and there is currently no sustainable alternative that can replace it [2]. The safety and availability of blood and its derivatives for transfusion use require the involvement of voluntary, unpaid, and carefully selected donors [3]. In Italy, individuals must be at least 18 years old to donate blood and plasma, as minors are not permitted to do so, even with parental consent [4]. Data from the Italian Health Ministry and National Blood Centre, updated in 2023, show that the number of blood donations is increasing (1,677,698 people) compared to previous years, but it is still inferior to the pre-pandemic years [5]. Notably, the number of young donors in 2023, aged 18 to 35, increased compared to 2022 (492,059 people, +1.3%). The aging of the population is evident in the data, as the number of donors older than 46 years changed from 650,202 to 787,156 between 2012 and 2021 [6]. Recruiting and retaining young people as blood donors is increasingly important to ensure an adequate supply of blood products for healthcare services [7]. Enhancing education and awareness about the need for blood within the population can empower even those unable to donate to become active advocates for blood donation. Research has shown that individuals with higher levels of education are more likely to donate blood, underscoring the importance of instilling proper knowledge on the topic at a young age [8]. In addition, it is crucial to motivate first-time donors to become usual donors [2, 3, 5, 6–8]. This study, conducted in collaboration with the Transfusion Centre of the Giovan Battista Grassi Hospital in Rome, primarily aimed to evaluate whether the lecture increased students' willingness to donate blood. A secondary objective was to validate the Italian translation of two questionnaires assessing the prevalence and motivation for blood donation among young people. To achieve these objectives, the study analyzed students' perspectives and knowledge on the topic at two key points: before and after the designated lectures.

MATERIALS AND METHODS

Study design

The study employed an observational design with a prospective, cross-sectional approach and a pre-post analysis.

Sampling

A non-probabilistic convenience sample was drawn from the senior students at the "Democrito" high school in Rome during the 2022–23 scholar year, allowing for the inclusion of all students in their final year. The eligibility criteria were: age 18 years or more, willingness to participate, provision of informed consent and acceptance of personal data processing. All eligible students were invited to take part in the project, with the decision whether to participate or not based on their own will.

Ethical approval

The students' participation was initially approved by the high school headmistress. Later, a school communication was released [9]: it clearly and concisely reported the nature of the study, its phases, the goals, and the students' involvement. This notification served as informed consent for students who were of legal age (18 years or older), enabling them to make an informed decision about their participation in the current study. Students had the freedom to withdraw at any given moment and for any reason. The high school teachers involved were notified in advance by the school about the planning of the study and provided their collaboration.

The protocol of the study was submitted to the Ethics Committee of the A. Gemelli Hospital in Rome, to confirm the validity of the protocol and ensure the protection and safety of the students involved. The study was approved with Opinion ID 5671/2023 [10].

Choice and Italian translation of the questionnaires

To find the most suitable questionnaires, we conducted a literature review on the PubMed/Medline database in October 2023. The research question "Is there a tool that assesses knowledge and motivations for/against blood donation?" led to the development of the following two search strings: 1) (high school students) AND (inquiry instrument) AND (information on blood donation; motivation to donate blood). 2) (population) AND (survey) AND (blood donation).

Subsequently, we inserted these filters: Publication date - 10 years; Human species; Language – Italian, English. With these criteria, the search produced 443 articles. We then performed a title and abstract screening, and then a full text screening according to the chosen inclusion and exclusion criteria (Table 1).

Based on the characteristics of the studies obtained from the literature review, we chose the two questionnaires to translate and use considering their construction, the variables investigated, the ease of compilation, the method of administration, and the population sample identified for the study. The first chosen questionnaire was selected from a German study by Greffin et al. in 2021 that investigated knowledge and prevalence of blood donation [11], and the second one was selected from a Spanish study about motivations behind donating by Romero-Dominguez et al [12]. Since the administration of the questionnaires was for young people (18-35 years old)

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Table 1. Literature review for the questionnaire choice: inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
— Full text available; — Survey; — Availability or possibility to rebuild the questionnaires; — Blood donation; — Male and female population; — Knowledge of and motivation for blood donation.	— Covid-19 studies; — Survey on infectious diseases, epidemics or pathologies in general; — Participation of health workers only; — Organ donation; — Study population chosen based on sexual orientation, ethnicity or migration status; — Data managing methods; — Studies on blood tests levels.

Table 2. CVI calculation per Item and of the whole questionnaires

1st questionnaire (Greffin, Schmidt, Schönborn, Muehlan, 2021)		2nd questionnaire (Romero-Domínguez, Martín-Santana, Sánchez-Medina, Beerli-Palacio, 2021)	
Item I. have you ever donated blood?	CVI =0.96	**Item V**. Solidarity	CVI =0.95
Item II. Based on your answer, we would like you to describe in your own words. What, so far, your personal reasons were for donating blood or not? Please try to answer this question as accurately as possible.	CVI =1	**Item VI**. Health benefit	CVI =0.78
Item III. Do you intend to donate blood within the next 12 months?	CVI =0.94	**Item VII**. Appreciation	CVI =0.90
Item IV. Based on your answer, we would like you to describe in your own words. What are your personal reasons for donating blood or not in the future? Please try to answer this question as accurately as possible.	CVI =1	**Item VIII**. Marketing stimuli	CVI =0.97
		Item IX. Social approval	CVI =0.94
Tot 1st questionnaire	CVI =0.975	Tot 2nd questionnaire	CVI =0.908

in Italy, it was necessary to translate them into Italian; for this purpose, we used the WHO Guidelines on Translation "Process of translation and adaptation of instruments" [13]. The content validity index (CVI) was calculated following WHO guidelines (Table 2).

The translation was performed only after receiving the authors' permission [11,12]. The translation of the questionnaires from English to Italian was not literal, but priority was given to the logical concept of the sentence. The sentences were short, simple and essential; we avoided scientific terms considering the target population. An Expert Committee of six professionals (a medical doctor in psychiatry, a medical doctor in hematology, a nursing university teacher, a community nurse, an Italian literature and Latin teacher and a psychologist) checked the content validity, using a 5-point Likert scale measurement (1=completely unrelated; 5=strongly related) [14, 15] based on the applicability of content and clarity of phrasing.

Using Likert scale assessments [14, 15], the CVI was calculated for each item (question), as the number of scores >3 divided by the total number of experts; a value >0.7 was considered acceptable. The CVI of the entire questionnaire (united CVI) was estimated by calculating the average content validity indices of all items. The scores were 0.975 and 0.908 respectively. To ensure the correct interpretation of the concepts, a translation check was carried out starting from Italian and returning to English using the Word Reference platform. This step showed no change in questionnaire content or meaning. The request for pre-testing of the translated questionnaires was submitted

to the Ethics Committee of the Agostino Gemelli University Hospital (Prot N. 0014209/23) [10]. The pre-testing was carried out on a representative sample of the population. Once the survey was complete, the participants were interviewed to evaluate their actual understanding of the topic covered, of the correct formulation of the sentences and any misunderstanding due to the translation process. None of the students had difficulties in understanding or answering the questions and we did not receive complaints about the clarity, simplicity and expression of questions and answers in Italian. So, the translation was found suitable and adequate for the objective of this study. The Italian translation of the questionnaires is present in Table 3.

Table 3. Italian translation of the questionnaires

Questionario 1: *(Greffin, Schmidt, Schönborn, Muehlan, 2021)*
1. Hai mai donato il sangue? No, non ho mai pensato di donare il sangue. No, ma ho pensato di donare il sangue. No, ma ho provato a donare il sangue e non mi è stato permesso. Si, ho già donato il sangue. 2. Basandoti sulla risposta precedente descrivi in poche parole, quali sono le ragioni personali per cui HAI o NON HAI donato il sangue? 3. Pensi di donare il sangue nei prossimi 12 mesi? Assolutamente no Probabilmente no Preferirei di no Preferirei di si Probabilmente si Assolutamente si 4. Basandoti sulla risposta precedente descrivi in poche parole, quali sono le motivazioni per cui DONERAI o NON DONERAI il sangue in futuro?
Questa parte propone una scala olistica ed integrativa sulle motivazioni per cui eventualmente donare il sangue. *(Romero-Domínguez, Martín-Santana, Sánchez-Medina, Beerli-Palacio, 2021)* Per favore segna se una tra le seguenti motivazioni potrebbe motivarti ad aumentare il numero di donazioni che fai annualmente (scegliere un opzione per gruppo)
1. Solidarietà Solidarietà umana, aiutare gli altri o salvare vite. Adempiere doveri sociali o doveri morali nell'aiutare le altre persone. Donare il sangue non costa niente. Visto che il sangue non può essere creato artificialmente, dobbiamo collaborare. Soddisfazione personale derivata dall'aiutare gli altri. Donare il sangue mi fa sentire necessari* e di aiuto per la società. Eventualmente io o i miei familiari potremmo avere bisogno di sangue in futuro. 2. Benefici per la salute E' bene per la mia salute. Avere i risultati degli esami del sangue. Sapere se ho una malattia infettiva. Ricevere pareri medici sulla mia salute. 3. Apprezzamento Ricevere regali simbolici per avere donato il sangue (magliette, spille, asciugamani, tazze, etc). Ricevere premi simbolici per la mia storia di donatore di sangue. Avere 1-2 ore di tempo libero da lavoro (scuola) per andare a donare il sangue. Acquisire il riconoscimento sociale associato all'essere un regolare donatore di sangue (eventi pubblici, attestati, medaglie, certificati, etc). 4. Stimoli di marketing Una chiamata urgente per la donazione di sangue. Vedere o sentire una campagna pubblicitaria in TV, radio o social media. Ricevere una chiamata o messaggio dal centro di donazione sangue. Conoscere la testimonianza di persone che hanno ricevuto una trasfusione di sangue. Autoemoteche vicino casa, lavoro/scuola o in luoghi affollati. 5. Approvazione sociale Gli altri avranno una buona opinione di me. La mia religione o credenze mi incoraggiano a donare. Donare il sangue è una tradizione di famiglia.

Validation of the questionnaire

The sample size was determined based on the final number of items included in the questionnaire. For exploratory factor analysis and internal reliability estimation, a rule of thumb frequently adopted in the literature is a minimum ratio of 5 participants per item (31-33) [16-19]. Although there is no universally agreed threshold for sample size, this ratio is commonly used as a reference in the preliminary stages of psychometric validation, especially when working with new instruments. As Osborne and Costello [18] point out, "a minimum subject to item ratio of at least 5:1 is recommended in exploratory factor analysis," while recognizing that higher ratios are desirable, when possible, based on the characteristics of the data and the expected factor structure. Considering these indications and the logistical limitations associated with the fact that only students in their final year of high school could be involved, given the requirement to be of legal age to donate blood, the use of the minimum ratio appeared valid. In our study, the questionnaires included 4 questions and 1 question with 5 items, respectively. A 5-item questionnaire would therefore require the participation of at least 25 subjects, a criterion that was fulfilled in our study.

Phases of the study

The study consisted of several phases, and students were free to engage in any of them based on their preferences and their attendance at school on the designated days.

The following phases were accomplished.

Phase 1. Administration of two surveys on prevalence and motivation for blood donation among young adults

The two surveys consisted of the two selected and translated questionnaires about knowledge [11] and motivations [12] for blood donation. The two surveys were administered twice: before (October 2022) and after (December 2022) the lectures (November 2022). Senior students received an email containing a QR code that linked to the two surveys. Responses were collected using Google Forms. An external collaborator was assigned the task of gathering responses and generating a unique numerical identification for each student, enabling us to access only the birth date and gender without disclosing names. Such pseudonymized data were then transferred to Excel (Supplementary material 1).

Phase 2. Lectures about blood donation

The approach included sensitizing and informing students about blood donation through three educational/motivational interventions held at the high school, despite our objective not being to assess any improvement in students' knowledge. The school conference room hosted the lectures, which were conducted over three days by a nurse. During these sessions, digital materials (slides), informational brochures, and custom pins were distributed. Topics covered included the nature of blood and its irreplaceability, benefits of blood donation for donors, recipients and community, current blood donation statistics in Italy, eligibility criteria for donors, temporary disqualifiers, the donation process, and donation locations. Although the collection of such information was not the main objective of this study, an observer recorded additional data on students' reactions during the lectures. These observations were useful for assessing students' actual level of engagement. This was done using an observational chart with a 5-point Likert scale (1=never; 5=always) to classify behaviors. The elements assessed included: "concentration," "active participation (questions, comments, observations)", "external interruptions", "distractions (cell phone use, whispering) or lack of interest" and "indifference". Two additional polls were conducted: one pre-lecture to gauge initial interest, and one post-lecture to assess perceived usefulness.

The initiative also emphasized the importance of maintaining their own health and promoting a healthy lifestyle

Phase 3. Blood donation at the Transfusion Centre.

Organizing specific days at the Transfusion Centre served to involve and encourage young adults to begin regular blood donation. All senior students who wished to donate blood and met the eligibility criteria were invited to participate in designated donation days. These criteria, explained during the lectures, included being over 18 years old, weighing more than 50 kg, being in good health (free from flu, colds, or similar conditions, and not taking medications such as cortisone, antihistamines, antibiotics, or anxiolytics), and maintaining a healthy lifestyle (no tattoos or piercings, no use of narcotics or alcohol, and no sexual promiscuity) [20]. To facilitate student participation, several measures were implemented: a notification was sent to all teachers to avoid scheduling class assignments or tests on donation days; students were reassured that the Transfusion Centre would provide a certificate to justify their absence from school, which also counted towards school credits; and the Volunteer Association announced structured scholarship opportunities.

Statistical analysis

Descriptive analysis of qualitative variables was performed, presenting data as absolute frequencies (number of subjects) and percentage frequencies. To assess changes in questionnaire responses before

and after the lessons, a McNemar test (a statistical test used on paired nominal data) was applied to compare response percentages. It is applied to 2 × 2 contingency tables with a dichotomous trait, with matched pairs of subjects, to determine whether the row and column marginal frequencies are equal. Statistical analyses were conducted using Stata software, version 18 (StataCorp LLC, College Station, TX, USA).

We attempted to use a statistical analysis to determine whether our health promotion lectures affected students' attitudes toward blood donation and their propensity to donate. To determine whether or not the lectures had a positive impact on the students, matching the answers was required. Although 135 students answered the pre-lesson survey and 119 answered the post-lesson survey, the matching of unique student codes yielded only 54 corresponding responses.

We took into consideration the responses to Greffin et al.'s [11] first two questions during this process: "Have you ever donated blood?" (Question 1) and "Do you intend to donate blood within the next 12 months?" (Question 2). The first question had four possible answers: 1) No, I can't imagine donating blood yet; 2) No, although I can imagine donating blood; 3) No, but I have already tried donating blood and I was not allowed to donate; 4) Yes, I've already donated blood. The first response was regarded as "Negative," but the second, third, and fourth responses were included as "Positive" due to the small sample size. There were six possible responses to the second question: 1) Definitely not; 2) Maybe not; 3) Would rather not; 4) Would rather; 5) Probably; and 6) Definitely. The first three responses were included as "Negative" due to the small sample size, while the fourth, fifth, and sixth responses were regarded as "Positive". After analyzing the total responses, the students were also separated based on their sex. For T1 we indicated the pre-lessons questionnaire, while for T2 we indicated the post-lessons questionnaire.

RESULTS

As shown in Table 4, the students who answered positively at T1 and confirmed their positive answer at T2 were 28, while there were 3 students who answered positively at T1 but changed their answer into a negative one at T2. Instead, there were 10 students who answered negatively at T1 and confirmed their negative answer at T2, while there were 13 students who answered negatively at T1 but changed their answer into a positive one at T2. With this data we built the 2x2 contingency table for the McNemar test as following.

In the pre-lesson questionnaires, 31 out of 54 students (57.4%) answered positively, while in the post-lessons questionnaire there were 41 out of 54 positive answers (75.9%), with a difference of 18.5%. The test gave a p-value of 0.0213, statistically significant. This means that the lectures had a positive impact on students' perspectives on the subject, making them understand the importance of being a blood donor.

In Table 5, we tried to apply the McNemar test to the male population of our study. Since the p-value would not be reliable with this very small sample, we provided only a description with absolute numbers. As we can see, there were 10 students who answered positively at T1 and confirmed their positive answer at T2, while only 1 student answered positively at T1 but changed his answer into a negative one at T2. Instead, there were 8 students who answered negatively at T1 and confirmed their negative answer at T2, while there were 7 students who answered negatively at T1 but changed their answer into a positive one at T2. But subgroup analyses are purely descriptive and should not be interpreted as statistically meaningful.

In Table 6, we tried to apply the McNemar test to the female population of our study. Since the p-value would not be reliable with this very small sample, we provided only a description with absolute numbers. As we can see, there were 18 students who answered positively at T1 and confirmed their positive answer at T2, while 2 students answered positively at T1

Table 4. McNemar test for overall students (N=54)

		POST-LECTURES	
		POSITIVE	**NEGATIVE**
PRE-LECTURES	POSITIVE	28	3
	NEGATIVE	13	10

McNemar's test: Difference: 18.52%; 95% Confidence Interval: 4.87% to 32.17%; p-value: 0.0213

Table 5. Absolute frequency in questionnaire responses before and after the lessons (male subjects; N=26)

		POST-LECTURES	
		POSITIVE	**NEGATIVE**
PRE-LECTURES	POSITIVE	10	1
	NEGATIVE	7	8

Table 6. Absolute frequency in questionnaire responses before and after the lessons (female subjects; N=28)

		POST-LECTURES	
		POSITIVE	**NEGATIVE**
PRE-LECTURES	POSITIVE	18	2
	NEGATIVE	6	2

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but changed their answer into a negative one at T2. Instead, there were 2 students who answered negatively at T1 and confirmed their negative answer at T2, while 6 students answered negatively at T1 but changed their answer into a positive one at T2. Since the p-value would not be reliable with this very small sample, we provided only a description with absolute numbers. But subgroup analyses are purely descriptive and should not be interpreted as statistically meaningful.

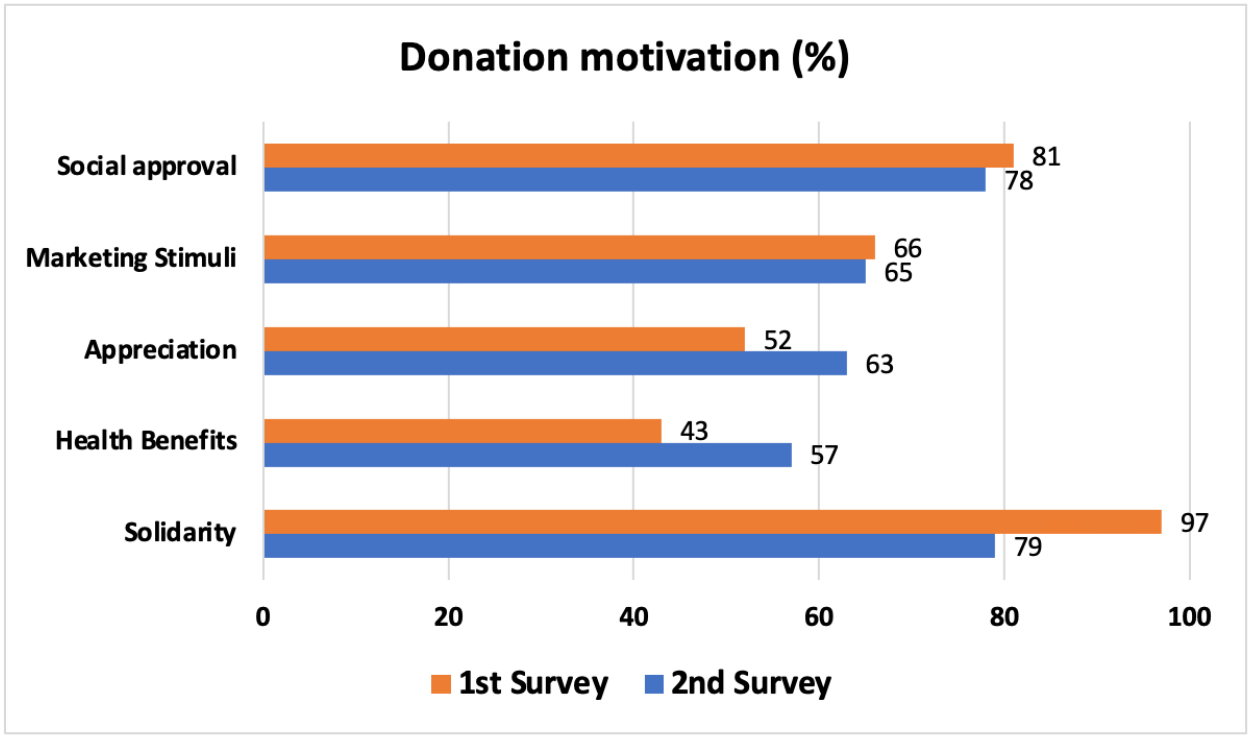
The overall analysis shows that, of the students who responded to the questionnaire given prior to the lessons, 31 out of 54 (57.4%) gave a positive response to the first question, while 23 gave a negative response. Out of the 31, 14 gave a negative response to the second question, while 17 gave a positive response as well. Only 14 students responded negatively to the questionnaire given after the lessons, while 41 out of 54 students (75.9%) responded favorably to the first question. Of these 41 students, 30 also responded favorably to the second question. Ultimately, four of these thirty students donated blood.

The motivations for donating blood included solidarity, health benefits, appreciation, marketing stimuli, and social approval (figure 1). The most common reasons were "human solidarity, helping others, or saving lives" (1st survey: 97 students; 2nd survey: 79 students), "getting medical advice about my health" (1st survey: 43 students; 2nd survey: 57 students), "receiving symbolic gifts for donating blood" (1st survey: 52 students), "having 1-2 hours of free time at work to donate blood" (2nd survey: 63 students), "knowing the testimony of people who have received a blood transfusion" (1st survey: 66 students; 2nd survey: 65 students), and "others will have a good opinion of me" (1st survey: 81 students; 2nd survey: 78 students). Survey results showed that three-quarters of the students wanted to participate in the lectures, and 107 out of 119 students found them helpful. The observer, using a Likert scale (1=never; 5=always), reported that all classes showed "concentration" and "active participation" scores between 3 and 5, while "distraction" and lack of interest scored between 1 and 3. Ultimately, 32 students, representing 17% of the total surveyed, signed up to donate blood on the designated days, but only 22 were eligible and able to proceed with the donation.

DISCUSSION

In Italy, blood shortages present a significant public health challenge. Understanding young people's knowledge and attitudes about blood donation can help identify effective strategies to increase their motivation to donate. Young adults are considered a crucial potential pool of blood donors; thus, insights into their perceptions of blood donation can aid in long-term recruitment efforts. The results of this study indicate that educational interventions can positively influence students' attitudes towards blood donation. The McNemar test, applied to the overall student population, revealed a statistically significant shift towards positive responses regarding both past blood donation experience and future intentions following the

Figure – Questionnaire on motivations on blood donation (Romero-Dominguez L., Mar-tin-Santana J.D., Sanchez-Medina A.J., Beerli-Palacio A., 2021)



educational lessons (p-value = 0.0213). This suggests that the lectures effectively conveyed the importance of blood donation and prompted students to reconsider their stance on the subject. While the overall impact of the intervention appears positive, the analysis of responses by sex presents a more nuanced picture. Although the small sample sizes for both male and female groups preclude reliable statistical analysis using the McNemar test, the descriptive data from the 2x2 contingency tables offer some insights. Both male and female students demonstrated a trend towards increased positive responses after the lectures. However, the magnitude of this shift varied, with a seemingly more pronounced change among female students. Having said that, various literature evidence show how donating blood is more common among males, because many women avoid donating due to perceived ineligibility or past rejections due to low body weight and anemia [21]. They recommend increasing female donor numbers through iron supplementation and personalized monitoring [22–25]. This approach should be supported by clear information, reassuring women that deferral is temporary and encouraging them to return once their hemoglobin levels stabilize [26]. Bani M. and Giussani B. [27] reported that perceived anxiety correlates with adverse reactions and impacts the likelihood of female donors returning [27, 28]. Strategies to reduce adverse reactions include fluid intake before donation, muscle tension exercises, audiovisual materials, and social support [27]. Further research with larger sample sizes is needed to explore potential gender-specific responses to blood donation education. It's important to note that a positive shift in attitude does not necessarily translate into actual blood donation behavior. Despite a significant increase in positive intentions, only four out of the 30 students who expressed positive intentions post-lectures ultimately donated blood. This discrepancy highlights the gap between intention and action, a common phenomenon in health behavior research. Several factors contribute to this gap, including logistical barriers, fear of needles, unforeseen personal circumstances, or anxiety [28]. The context in which blood donation is proposed can significantly impact anxiety, attitudes, subjective norms, self-efficacy, and intention to donate [29, 30]. Tailored brochures can reduce donor anxiety according to a study by Newman B. et al. [31]. Future studies could investigate these factors to identify potential strategies for bridging the intention-behavior gap and increasing actual blood donation rates. The survey results also shed light on the motivation behind students' decisions to donate blood. Altruistic motives, such as "human solidarity" and "helping others," emerged as prominent factors. However, other motivations, including health benefits, symbolic gifts, and social approval, also played a role. Understanding these diverse motivations can inform the development of targeted recruitment campaigns that appeal to a broader range of potential donors. Finally, the positive feedback received from students regarding the lectures themselves underscores the importance of engaging and informative educational interventions in promoting blood donation. The high levels of concentration and active participation observed during the lectures suggest that the educational content was well-received by the students. However, the negative reactions observed in two classes highlight the need for ongoing evaluation and refinement of educational materials to ensure their effectiveness across diverse student populations.

Despite the challenges inherent in conducting innovative research within a real-world school environment, our study is distinguished by several key strengths. First and foremost, the project's innovative approach—integrating a novel educational intervention into the established routines of a high school—demonstrates both creativity and adaptability. Successfully implementing this intervention amidst the complexities of school scheduling highlights the feasibility of such programs and sets a valuable precedent for future health promotion initiatives in educational settings.

Additionally, while the study focused exclusively on senior students from a single high school, this concentrated approach enabled us to engage deeply with participants, maintain rigorous control over the intervention, and ensure consistent delivery of educational content. This focus facilitated close monitoring of student responses and allowed for a thorough evaluation of the intervention's impact.

Although the sample size was limited, with many students participating in only one questionnaire, the study nonetheless achieved statistically significant results. These findings provide compelling preliminary evidence of the intervention's positive effect on students' intentions to donate blood, offering a strong foundation for future, larger-scale research. The project's design and demonstrated outcomes present a scalable and adaptable framework that can be extended to other schools, underscoring its potential to promote blood donation among young people on a broader scale.

While further studies are necessary to confirm the generalizability of these results, our research highlights important factors for success and points to areas for future improvement. Subsequent investigations could examine the specific elements that influenced student reactions and refine strategies to enhance the effectiveness of blood donation education.

CONCLUSION

This study provides valuable insights into the attitudes and motivations of young adults toward blood donation. The educational intervention appears to have positively influenced students' perceptions, particularly among female participants. However, bridging the intention-behavior gap remains a significant challenge. Ongoing efforts to address logistical barriers, reduce

anxiety, and leverage diverse motivations are crucial for enhancing blood donation rates among the youth. Further research with larger, more representative samples is needed to validate these findings and explore gender-specific strategies for promoting blood donation.

AUTHOR CONTRIBUTIONS

Conceptualization, S.R., S.M. and P.L.; methodology, V.D.V., D.P.M. and M.C.N.; software, D.P.M.; validation, P.L.; formal analysis, V.D.V. and D.P.M.; investigation, S.R.; data curation, V.D.V., B.C., L.M. and S.R.; writing—original draft preparation, S.R., R.M. and S.M.; writing—review and editing, V.D.V., D. P. M. and D. P.; supervision, V.D.V., P.L. and G.D.; project administration, P.L.. All authors have read and agreed to the published version of the manuscript.

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INSTITUTIONAL REVIEW BOARD STATEMENT

The Ethics Committee of the Agostino Gemelli University Polyclinic Foundation was involved for the development of this study (Delibera ID 5671. Prot N. 0014209/23 del 08/05/2023). The research was conducted ethically, with all study procedures being performed in accordance with the requirements of the World Medical Association's Declaration of Helsinki.

INFORMED CONSENT STATEMENT

Written informed consent was obtained from each participant/patient for study participation and data publication.

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

COVERING LETTER

The manuscript has been seen and approved by all authors, it is not under active consideration for publication, has not been accepted for publication, nor has it been published, in full or in part (except in abstract form).

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Association between Hand Grip Strength and Mortality: The North West, South Africa Prospective Urban Rural Epidemiology (PURE) Study

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SUMMARY

Hand Grip Strength (HGS) is employed in epidemiological contexts to measure muscle strength because it is inexpensive, easy to perform and interpret. Population-based investigations use protocols that incorporate HGS measurements in health-status evaluations. Our aim was to investigate the association of HGS with all-cause mortality in a South African population. Methods This study was based on the South African leg of the Prospective Urban and Rural Epidemiology (PURE) study, a community-based, prospective cohort study. This work was based on baseline HGS collected in 2005. Deterministic linkage to the mortality was performed using death status in 2018. The Cox regression was applied to investigate all-cause mortality risk in relation to HGS tertiles. A non-linear dose response analysis has been applied to investigate the shape of the relation between HGS and all-cause mortality risk. All the results were validated by numerous sensitivity analyses. Results Our work included 1 251 participants with a median age of 47 years (5th95th quantile range 36, 67) and 59.6% (n 746) of participants were women. During a median follow-up of 13.2 years, 374 deaths from all causes occurred. We observed a hazard ratio of 0.80 (95% CI = 0.61, 1.05) and 0.61(95% CI = 0.44, 0.85) decreased risk of all-cause mortality for the 2nd and 3rd tertiles of dominant hand grip strength compared to the 1st. A similar risk reduction was observed for the non-dominant hand. A linear monotone decreasing relation between HGS and all-cause mortality risk was reported. Conclusions HGS is inversely associated with mortality risk and can be used to predict mortality risk in the South African population.

Keywords: Hand grip; Mortality; Sub Saharan Africa; PURE study.

INTRODUCTION

Hand grip strength (HGS) is the force that the host of muscles in the hand and forearm can produce [1]. Although hand grip strength indicates muscular strength in the hand and forearm, it is a proxy of upper body muscle strength [2]. Measures of HGS are employed in clinical and epidemiological contexts to measure

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muscle strength because the test is inexpensive, easy to perform and easy to interpret [3,4]. Population-based investigations, such as the UK Biobank study, use protocols that incorporate HGS measurements [5].

Data from HGS assessments has been used to assess health-related issues [5–11]. Additionally, HGS gives an overall indication of muscle health and possible susceptibility to muscular disorders [12,13]. Muscle strength and endurance decrease due to aging and development of chronic diseases, resulting in a loss of functional performance [14]. Similarly, sickness and lifestyle factors affect muscular strength because muscle atrophy and lack of optimal nutrition contribute to the deterioration of muscle mass and muscle activation [13,15]. The lack of muscle mass and concomitant decrease in functional ability and performance, connects HGS to many other health-related factors, such as bone mineral density, nutritional status, and sleep related conditions [2]. Thus HGS is related to overall health in individuals and is influenced by lifestyle and daily living activities [4,16–18].

There is abundant information on the association between HGS and mortality on populations in high income countries (HIC). However, such information is scarce in low- and middle-income countries (LMIC). The aim of this study was to investigate the association of HGS with all-cause mortality in a South African population. We first investigated the association between dominant and non-dominant HGS with all-cause mortality at 13 years' follow-up. Afterwards, we investigated the shape of the association between HGS and all-cause mortality risk using a non-linear dose-response analysis. Numerous sensitivity analyses and adjusted models were adopted to exclude potential biases and reversal causation. Finally, we used an internally cross-validated analysis to investigate if HGS from dominant hand is a better all-cause mortality predictor than HGS from non-dominant hand.

METHODS

Study design

This research study is part of The Prospective Urban and Rural Epidemiology (PURE) study which is a community-based multi-country longitudinal prospective cohort research study conducted to investigate the association between risk factors and various health outcomes [3]. Overall, 27 countries are involved in the PURE study. This study is based on data collected on a random stratified sample of 6,000 randomly selected households in the North West Province in South Africa. The urban stratum was defined by established townships near a large city, and the rural stratum was defined by tribally governed communities [19]. Baseline data were collected in 2005, the present study is based on full covariate information about 1,251 participants; 622 rural and 629 urban participants.

Data collection

Trained field workers used a standardized questionnaire to interview at least one household member for personal details and additional characteristics regarding the household [19]. Self-reported demographics, cardiovascular risk factors, comorbidity disorders, education levels, work position, physical activity levels, and dietary habits were collected through questionnaire-based interviews. A customized questionnaire was used to obtain data on prescribed medicines, alcohol, and tobacco use.

The Baecke physical activity questionnaire [20] is a short questionnaire for the measurement of habitual physical activity in epidemiological surveys. The questionnaire includes a total of 16 questions classified into three domains: work, sports, and non-sports leisure activity. Each domain has several questions scored on a five-point Likert scale, ranging from never to always or very often [20] . It defines three levels of occupational physical activity, namely low level (clerical work, driving, shop keeping, teaching, studying, housework, medical practice and most other occupations with a university education), middle level (factory work, plumbing, carpentry, farming) and high level (dock work, construction work, sport). Similarly, sports are categorized into three levels: low level (billiards, sailing, golf), middle level (badminton, cycling, dancing, tennis) and high level (boxing, rugby, football, rowing). A sport participation score is calculated from the intensity factor, the number of times per week participating in that type of sport and the proportion of the year in which the sport is played. Indices of physical activity for three dimensions, namely occupational physical activity, sport during leisure-time and physical activity during leisure time, excluding sport, can be established using the Baecke questionnaire (BQ). Test-retest reliability of the work index, sport index and leisure-time index varies between 0.74 and 0.88. The questionnaire can be used for the various socio-economic classes in the general population. The questionnaire has been used in the assessment of physical activity of study participants between the ages of 20 and 70 years and significant correlation coefficients ranging from 0.76 to 0.93 were found in reliability testing [20].

The Omron HEM-757 equipment (Omron Healthcare, Kyoto, Japan) was used to measure blood pressure with subjects in the supine position for at least five minutes. Hypertension is a systolic or diastolic blood pressure equal to or greater than 140mmHg systolic or 90mmHg diastolic blood pressure, as per the 2018 ESC/ESH guidelines [21]. Height was measured with a stadiometer and weight with a digital scale and used to calculate body mass index (BMI) in kg/m^2. HGS was measured by trained exercise professionals with a Jamar dynamometer, using a standardized protocol [3]. Three measurements to the nearest kilogram were recorded from the participant's dominant and non-dominant hand, the highest value was considered for the analysis.

Mortality data, as recorded on the participants' death certificates provided by Statistics South Africa dated 2018, was the outcome considered. The study adhered to the revised Helsinki Declaration and was approved by the North-West University Health Research Ethics Committee for Humans with ethics number 04M10 and NWU-00016-10-A1. All involved participants signed informed consent forms for data processing and handling. Participants were free to withdraw from the study at any time.

Statistical methods

Data description was performed by median and 5th to 95th percentile range for continuous variables, counts and percentages were used for categories. The Cox proportional hazards model was used to estimate the hazards of all-cause mortality by tertiles of dominant and non-dominant HGS and by one standard deviation increase. To this aim, the Cox proportional hazard model had sex, 10-years age categories and locality (rural or urban) as strata factors. The hazard ratios (HRs) for one standard deviation increase were performed after transforming the HGS variable with Blom's transformation, resulting in a normal standardized variable [22]. Moreover, all analyses were adjusted for medication use, socio-economic status (cross categories of employment and education above grade 8th), hypertension or use of anti-hypertensive medication, former or current tobacco use, former or current alcohol use, diabetes, any prevalent diseases such as HIV or TB, cardiovascular or respiratory diseases or cancer, physical activity index according to the Baecke questionnaire, and BMI. Supplementary analyses were performed excluding participants with positive baseline HIV or tuberculosis, cardiovascular diseases, and cancer. Sensitivity analyses were conducted excluding participants who experienced death in the first year of observation. A non-linear dose response analysis was performed to investigate the shape of the relation between HGS and mortality risk. To this aim, we used a restricted cubic spline with four knots placed at the 5th, 35th, 65th and 95th percentiles.

Finally, we used a Least Absolute Shrinkage and Selection Operator (LASSO) analysis to determine which of the dominant or non-dominant hand was the best predictor of all-cause mortality. Briefly, we divided our data frame into two equal subsets, a training and a test data frame. A first model was performed on the training data frame, afterwards the model was validated by a LASSO approach on the test data frame. Variable selection was performed by means of the optimal Lambda parameter of the LASSO model [23–25]. The Cox proportional hazard assumption of the risk proportionality was assessed by a model having a multiplicative interaction term between HGS and the log-transformed time [26]. All statistical tests were two-tailed with a type-I error rate of 5% (α = 0.05). The HRs were estimated using the PHREG procedure of the SAS software vers 9.4. The non-linear dose-response analysis was performed using the mkspline function of the STATA software vers. 14. The LASSO analysis was performed by a customized approach based on the glmnet package of the R software.

RESULTS

This study included 1,251 participants with a median age of 47 years (5th to 95th range = 36; 67), 59.6% (n = 746) of participants were women and 50.3% (n = 629) were from the urban area. During a median follow-up of 13.2 years, 374 deaths from all causes occurred. Regarding behavioural risk factors, 59.6% of the participants were tobacco users, 48.2% were alcohol consumers and the median Baecke physical activity index was 7.5 (5th to 95th range = 4.6; 10.2). Regarding the metabolic risk factors, 47.5% had hypertension, 6.2% had type two diabetes, the median BMI was 22.3kg/m^2 (5th to 95th range = 16.2; 38.5), and the prevalence of obesity (BMI > 30 kg/m^2) was 21.1%. When looking at baseline prevalent diseases, 4.5% of participants had infectious diseases (HIV and TB), 6.2% participants had cardiovascular diseases (CVD) and respiratory infections (RI), 0.3% had cancer, and 13.3% were using medication. Among all participants, 69.2% were educated above grade 8 and employed. For HGS, the median measurement for the dominant hand was 32.0 N (5th to 95th range = 20.0; 52.0) and 30.0 N (5th to 95th range = 18,0; 50.0) for the non-dominant hand. There were 374 deaths at the end of the follow up. The median age at baseline of those that died was 51.0 (5th to 95th range = 36.0; 72.0). Of the deceased participants, 46.5% were women and 59.9% were from urban areas. The behavioural risk factors for the deceased were: 69.8% were tobacco users and 62% of them were alcohol consumers. Regarding the physical activity index, the median was 6.6 (5th to 95th range = 4.3; 9.8). Regarding the metabolic risk factors, 56.7% of the participants were hypertensive and 7.2% had type 2 diabetes. The median BMI for those that died was 20.7 kg/m^2 (5th to 95th range = 15.6; 36.8). The prevalent diseases for the deceased were 8% for infectious diseases (HIV and TB), 6.2% for CVD and RI, and 0.3% for cancer. In addition, the cause of death was undetermined for about 70% of the cases. Results indicate 14.7% of the deceased were using some type of medication. Among the deceased participants, the majority had a job and education above grade 8 (78.1%). The baseline characteristics of all participants in the study sample are reported in Table 1.

We observed hazard ratio (HR) of 0.80 (95% CI = 0.61; 1.05) and 0.61, (95% CI = 0.44; 0.85) for risk of all-cause mortality for the 2nd and 3rd tertiles of dominant hand grip strength compared to the 1st tertile. Similarly, there was a HR of 0.65

Table 1. Baseline characteristics of the study sample

	All Participants n = 1,251	**Survivors n = 877**	**Deceased n = 374**
Age(years)	47.0 (36.0; 67.0)	46.0 (36.0; 65.0)	51.0 (36.0; 72.0)
Women	746 (59.6)	572 (65.2)	174 (46.5)
Urban	629 (50.3)	405 (46.2)	224 (59.9)
Educated and employed	866 (69.2)	574 (65.5)	292 (78.1)
Educated and unemployed	53 (4.2)	51 (5.8)	2 (0.5)
Uneducated and employed	238 (19.0)	185 (21.1)	53 (14.2)
Uneducated and unemployed	94 (7.5)	67 (7.6)	27 (7.2)
Smokers	746 (59.6)	485 (55.3)	261 (69.8)
Alcohol use	603 (48.2)	368 (42.0)	235 (62.8)
Hypertension	594 (47.5)	382 (43.6)	212 (56.7)
Type 2 Diabetes	78 (6.2)	51 (5.8)	27 (7.2)
Infectious diseases	56 (4.5)	26 (3.0)	30 (8.0)
CVD and RI	78 (6.2)	55 (6.3)	23 (6.2)
Cancer	4 (0.3)	3 (0.3)	1 (0.3)
Use of medication	166 (13.3)	111 (12.7)	55 (14.7)
Body mass index (kg/m^2)	22.3 (16.2; 38.5)	23.5 (16.8; 39.0)	20.7 (15.6; 36.8)
Physical Activity Index	7.5 (4.6; 10.2)	7.7 (4.7; 10.3)	6.6 (4.3; 9.8)
DHG (N)	32.0 (20.0; 52.0)	32.0 (20.0; 52.0)	32.0 (18.0; 50.0)
NHG (N)	30.0 (18.0; 50.0)	30.0 (20.0; 50.0)	30.0 (18.0; 50.0)

Notes. *Grade 8th was the threshold chosen for education, Infectious diseases: HIV and Tuberculosis (TB), CVD and RI: Cardiovascular Diseases and Respiratory Infections, DHG: Dominant hand grip, NHG: Non-dominant hand grip, N: Newtons (unit of measure)*

(95% CI = 0.49; 0.86) and 0.64, (95% CI = 0.46; 0.89) for all-cause mortality risk for the same analysis applied for the non-dominant hand. Moreover, we observed a HR of 0.75 (95% CI = 0.66; 0.86) for the dominant hand and 0.76 (0.66; 0.87) for the non-dominant hand for one standard deviation increase of HGS. After the exclusion of participants who died within the first year of the study, a HR of 0.82, (95% CI = 0.62; 1.09) and 0.66, (95% CI = 0.47; 0.93) for all-cause mortality risk was observed for the 2nd and 3rd tertile respectively compared to the 1st tertile for the dominant hand grip strength and HR of 0.70, (95% CI = 0.52; 0.94) and HR of 0.69, (95% CI = 0.49; 0.98) all-cause mortality risk for the 2nd and 3rd tertile compared to the 1st tertile, for the non-dominant hand. Additionally, we observed an all-cause mortality risk of 0.81 (95% CI = 0.69; 0.94) for the dominant hand and 0.83 (95% CI = 0.69; 0.98) for the non-dominant hand for one standard deviation increase for HGS.

The above results were confirmed by the sensitivity analysis performed regarding the exclusion of participants with infectious diseases, cardiovascular disease and/or respiratory infections, cancer, and those who were using any medication. When excluding the participants with any baseline infectious diseases, we observed a decreased all-cause mortality risk for the 2nd and 3rd tertile of dominant hand, likewise for the non-dominant hand grip strength, compared to the 1st tertile. Additionally, a HR of 0.75 (0.65; 0.86) for the dominant hand and 0.76 (0.66; 0.87) for the non-dominant hand for one standard deviation increase in HGS was observed after excluding participants with baseline infectious diseases. A decreased risk was also observed for the 2nd and 3rd tertile respectively compared to the 1st tertile for the dominant hand, similarly for the non-dominant hand, after the exclusion of participants with CVD and RI. Furthermore, we observed a HR of 0.74 (95% CI = 0.65; 0.84) for the dominant hand and 0.75 (95% CI = 0.65; 0.86) for the non-dominant hand for one standard deviation increase in HGS after exclusion of participants with baseline CVD or RI. After exclusion of participants with cancer, we observed an all- cause mortality risk reduction for the 2nd and 3rd tertile of dominant hand, correspondingly for the non-dominant hand grip strength with respect to the 1st tertile. In addition, when considering one standard deviation increase in HGS for the exclusion of participants with cancer, we observed a hazard ratio of 0.75 (95% CI = 0.66; 0.86) for the dominant hand and 0.76 (95% CI = 0.67; 0.87) for the non-dominant hand. Furthermore,

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we observed an all-cause mortality risk decrease for the 2nd and 3rd tertile respectively compared to the 1st after the exclusion of participants using any medication for the dominant hand as well as the non-dominant hand. Moreover, we observed a hazard ratio of 0.73 (95% CI = 0.63; 0.84) for the dominant hand and 0.75 (95% CI = 0.64; 0.86) for the non-dominant hand after the exclusion of participants using any medication for one standard deviation increase in HGS.

Complete HR values for the sensitivity analysis were given in Table 2. The graph of the non-linear dose-response relation between HGS and all-cause mortality risk appears as a monotone deceasing relation for both dominant and non-dominant hand. According to the Wald test of the spline terms, we observed a significant result for the linear terms while the quadratic and the cubic terms were not (Figure 1).

Table 2. Association between Hand grip strength and mortality for all causes

Total Sample				
Dominant hand	Cases	Persons-year	HR_1 (95% CI)	HR_2 (95% CI)
1st tertile	135	4614.3	1 (Ref.)	0.75 (0.66; 0.86)
2nd tertile	118	4754.8	0.80 (0.61; 1.05)	
3rd tertile	121	4686.4	0.61 (0.44; 0.85)	
Non dominant hand	Cases	Persons-year	HR_1 (95% CI)	HR_2 (95% CI)
1st tertile	142	4675.6	1 (Ref.)	0.76 (0.66; 0.87)
2nd tertile	98	4837.7	0.65 (0.49; 0.86)	
3rd tertile	134	4542.3	0.64 (0.46; 0.89)	
Exclusion of participants who died in the first year of observation				
Dominant hand	Cases	Persons-year	HR_1 (95% CI)	HR_2 (95% CI)
1st tertile	124	3715.63	1 (Ref.)	0.81 (0.69; 0.96)
2nd tertile	109	4017.63	0.82 (0.62; 1.09)	
3rd tertile	116	3853.92	0.66 (0.47; 0.93)	
Non dominant hand	Cases	Persons-year	HR_1 (95% CI)	HR_2 (95% CI)
1st tertile	129	3770.13	1 (Ref.)	0.83 (0.69; 0.98)
2nd tertile	93	4172.60	0.70 (0.52; 0.94)	
3rd tertile	127	3644.46	0.69 (0.49; 0.98)	
Exclusion of participants with infectious disease				
Dominant hand	Cases	Persons-year	HR_1 (95% CI)	HR_2 (95% CI)
1st tertile	125	4472.3	1 (Ref.)	0.75 (0.65; 0.86)
2nd tertile	111	4569.1	0.81 (0.61; 1.07)	
3rd tertile	108	4510.7	0.58 (0.41; 0.82)	
Non dominant hand	Cases	Persons-year	HR_1 (95% CI)	HR_2 (95% CI)
1st tertile	133	4510.8	1 (Ref.)	0.76 (0.66; 0.87)
2nd tertile	92	4721.2	0.65 (0.49; 0.87)	
3rd tertile	119	4320.1	0.62 (0.44; 0.88)	
Exclusion of participants with cardiovascular diseases and/or respiratory diseases				
Dominant hand	Cases	Persons-year	HR_1 (95% CI)	HR_2 (95% CI)
1st tertile	126	4286.0	1 (Ref.)	0.74 (0.65; 0.84)
2nd tertile	112	4424.1	0.80 (0.60; 1.06)	
3rd tertile	113	4451.2	0.57 (0.41; 0.80)	
Non dominant hand	Cases	Persons-year	HR_1 (95% CI)	HR_2 (95% CI)
1st tertile	134	4357.6	1 (Ref.)	0.75 (0.65; 0.86)
2nd tertile	89	4475.0	0.64 (0.48; 0.85)	
3rd tertile	128	4328.6	0.61 (0.44; 0.86)	

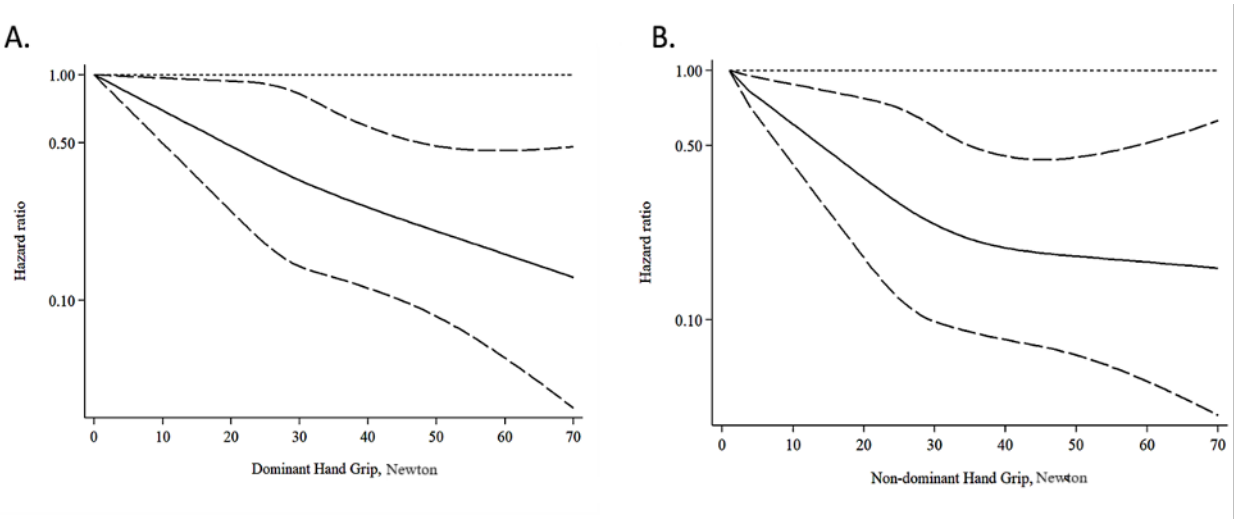
(*continued*)

Table 2. Association between Hand grip strength and mortality for all causes (continued)

Total Sample				
Exclusion of participants with cancer				
Dominant hand	Cases	Persons-year	HR_1 (95% CI)	HR_2 (95% CI)
1st tertile	134	4596.4	1 (Ref.)	0.75 (0.66; 0.86)
2nd tertile	118	4728.2	0.80 (0.61; 1.05)	
3rd tertile	121	4686.4	0.61 (0.44; 0.85)	
Non dominant hand	Cases	Persons-year	HR_1 (95% CI)	HR_2 (95% CI)
1st tertile	141	4657.6	1 (Ref.)	0.76 (0.67; 0.87)
2nd tertile	98	4811.2	0.66 (0.50; 0.87)	
3rd tertile	134	4542.3	0.64 (0.46; 0.89)	
Exclusion of participants using any medication				
Dominant hand	Cases	Persons-year	HR_1 (95% CI)	HR_2 (95% CI)
1st tertile	115	4043.9	1 (Ref.)	0.73 (0.63; 0.84)
2nd tertile	102	4172.9	0.76 (0.56; 1.03)	
3rd tertile	102	4065.9	0.56 (0.39; 0.81)	
Non dominant hand	Cases	Persons-year	HR_1 (95% CI)	HR_2 (95% CI)
1st tertile	121	4119.1	1 (Ref.)	0.75 (0.64; 0.86)
2nd tertile	82	4186.4	0.65 (0.48; 0.89)	
3rd tertile	116	3977.3	0.64 (0.45; 0.92)	

HR_1 = Multivariate adjusted all-cause mortality risk by tertiles, HR_2 Multivariate adjusted for one standard deviation increase

Figure 1. The non-linear dose-response analysis of HGS and all-cause mortality risk. The analysis of dominant and non-dominant HGS are reported on panes A and B, respectively. Units were measured in Newtons (N)



Finally, the LASSO analysis confirmed that dominant hand should be considered as a better predictor of mortality than the non-dominant hand. This result was confirmed after the exclusion of participants died during the first year of observation and after excluding subjects with baseline conditions such as cardiovascular disease and respiratory infections, cancer or using any medication.

DISCUSSION

This is the first long follow-up investigation reporting HGS in relation to mortality in Black South Africans. Firstly, we showed that the relation between HGS and all-cause mortality is robust after adjusting for numerous factors. This demonstrates that HGS was independently associated with all-causes mortality

risk thus confirming its application as a valid proxy of health status in this South African population. Moreover, we showed that HGS serves as an indicator of health status in the general population excluding any reversal causation as our results were consistent after the exclusion of participants who died within the first year of observation.

The validity of our results in the general population were confirmed by numerous sensitivity analyses corroborating the association between HGS and all-cause mortality without the direct influence of other comorbidities. We observed a monotone decreasing risk of all-cause mortality with increasing hand grip strength, for both the dominant and non-dominant hand. Using a cross validated LASSO model, we confirmed that HGS of the dominant hand is a better mortality predictor than the HGS measured in the non-dominant hand.

The above results agree with multiple other studies [3,7,13,27–30]. However, the majority of those studies were conducted on Caucasian populations or having a small percentage of black participants. The originality of the results presented in this study lies in the investigation of a population with a high bone mineral density compared to a Caucasian population. This study further presents the non-linear dose-response analysis for the HGS in the dominant and non-dominant hand in relation to mortality. We confirmed that the HGS from the dominant hand is preferably used as a quantifiable measure of muscle strength in epidemiological studies [2,31,32]. As previously stated, HGS is used to evaluate muscle strength as it reflects the strength of the whole body [14,17,33] which is a proxy of the overall health status of individuals. Therefore, muscle strength, as assessed by HGS, is indicative of muscle health and even of possible changes in physiological functioning [6,8,34,35]. Several studies reported the prospective association between muscle strength and mortality [12,27,36]. On the one hand, low HGS and muscle weakness, which is linked with low physiological function, have been associated with an increased risk of all-cause mortality [8,14,17]. Conversely, higher muscle strength is associated with reduced mortality [37,38]. Higher levels of HGS were associated with reduced risk of all-cause mortality in a study involving approximately 2 million healthy men and women [12]. Moreover, our results also agree with a study based on numerous mortality predictors showing that muscle strength is a reliable predictor of long-term mortality in initially healthy individuals [39]. Our findings are supported by numerous possible biological mechanisms. Increased strength could be an indicator of better early life nutrition as this can influence and affect mid-life muscle strength [2]. Additionally, mid-life strength may be affected by earlier life-style characteristics, such as physical activity [17]. In support of this, previous studies have shown that muscle strength is associated with physical activity and low mortality risk [10,35,40]. Furthermore, poor muscle strength could be an indicator of undetected or undiagnosed diseases in healthy adults [41]. Poor muscle strength in people with chronic conditions and diseases affects muscle protein synthesis [2,42,43]. Further, our dose response analysis indicates that the risk of mortality decreases linearly with increased HGS. This is consistent with results reported in a previous study where it was found that higher HGS was linearly associated with lower risk of all-cause mortality in middle- aged people [38].

The above results confirm numerous studies conducted on caucasian populations. On the other hand, different results may be expected due to the Black population possessing a higher bone mineral content and protein composition [44,45]. As a result of the physiological differences, the Black population is expected to have a higher muscle composition than the Caucasian population and therefore a higher HGS. However, our mean value of HGS for the dominant hand was 32.0 which is quite similar to that of the total PURE study (30.6 N) [3]. Other studies confirmed our results showing that the association between HGS and mortality remains independent after adjusting for different factors [13,28].

Strengths and limitations

Our study is based on robust statistical methodology based on the use of a multivariate adjusted model, thus addressing potential confounders. Additionally, our results are robust because we confirmed our findings by means of numerous sensitivity analyses. Moreover, using a nonlinear dose response we showed the linearity of the relation between HGS and all-cause mortality risk. The mortality in our population was expectantly high, i.e. about 30% of the subjects died after a 13-year follow-up in a population with a mean age of 47 at entry. This adds interest for the specificities of the population. However, our study is not free of limitations. A possible weakness is that our analysis is limited to the investigation of all-cause mortality and over 70% of deaths were due to undetermined causes. However, this does not affect the value of our study because all-cause mortality is an important epidemiological proxy of health. Moreover, considering specific mortality would have reduced the statistical power of our models resulting in many false negative results. However, its application in a South African target population had not been confirmed prior to this study. The accumulation of numerous scientific evidence about HGS and health, the existence of possible underlying mechanisms that explain this relation and finally, but not least, the evident dose-response association observed corroborates our results.

CONCLUSION

The observation that HGS is inversely associated with mortality risk is applicable in a South African

population irrespectively of potential physiological differences with Caucasians. We showed that this association is not affected when considering either the dominant or non-dominant hand. We also showed that HGS, and dominant HGS in particular, is a reliable proxy of general health in a population.

AUTHOR CONTRIBUTIONS

KM performed the statistical analyses and draft the first version of the manuscript, SK HM and MP were responsible for data collection and study design, MCM DS and CL provided technical support to manuscript writing and epidemiological interpretation of the data, CR conceived the work and the statistical analysis and supervised KM for the drafting of the first version of the manuscript. All authors revised and approved the last version of the manuscript.

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COMPETING INTERESTS

Authors have no conflicts of interest to declare

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Vending Machines and Youth Access to Cigarettes in Ireland: A Cross-sectional Study

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SUMMARY

Tobacco-related morbidity and mortality significantly adversely impact public health and well-being on a global scale. Most smokers start smoking before being legally of age to smoke. Cigarette vending machines are an acknowledged access route for underage smokers to access cigarettes. Using a convenience sample, this research uses an online survey to explore the willingness of adults to purchase a vending machine token for underage smokers. Data was collected from 599 participants. Over 12% of adults reported that they would buy such a token for a 17-year-old, while another 8.6% of respondents were unsure. Analysis revealed that smoking history and age were significant factors in predicting willingness to purchase a cigarette vending machine token for an underage smoker. As cigarette vending machines remain an access route for youths to cigarettes, this research supports the forthcoming legislation banning such machines in Ireland.

Keywords: Tobacco; Cigarettes; Cigarette Vending Machines; Youth; Health Promotion; Ireland.

INTRODUCTION

The use of tobacco continues to be a significant cause of avoidable illnesses and deaths around the world (1). According to the World Health Organization (WHO), it contributes to over 8 million deaths annually, significantly burdening healthcare systems and public health outcomes (2). The Global Burden of Disease Study highlights tobacco as a persistent risk factor across regions and age groups (3). However, it is not only the impact of tobacco on human health that should be considered. The adverse environmental damage caused by the tobacco industry has also been the focus of increasing attention (4,5).

In Ireland, smoking has long been a public health concern (6). Although significant efforts have been made in reducing prevalence through policy and education, smoking rates remain unacceptably high (7,8). The government set an ambitious goal to become smoke-free, defined as having a smoking rate below 5%, by 2025 (9). However, it is now clear that this target has not been met, as the smoking prevalence appears to have plateaued at 18% (8).

Youth smoking continues to present challenges and is acknowledged as a global public health problem (6,10). Data from the Healthy Ireland Survey 2024 indicate that individuals typically try their first cigarette at 16 years old and begin daily smoking at 18, with men starting slightly earlier than women (8). Findings from the Health Behaviour in School-aged Children (HBSC) Ireland survey similarly report that a sizeable proportion of school-aged adolescents have tried smoking at least once (11). Effective reductions in youth smoking must focus on both supply and demand, and enforcement is a crucial issue in addressing supply (10).

Cigarette vending machines have historically served as an access point to tobacco for underage smoking (12-15). Article 16 of the WHO's *Framework Convention on Tobacco Control* (FCTC) recommends a ban on vending machines or, at the very least, restrictions on youth accessibility to them (10, 15). In line with

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Statutory Instrument (S.I.) No. 42/2009 - Public Health (Tobacco) (Self Service Vending Machines) Regulations 2009, cigarette vending machines in Ireland must be activated by a token or card obtained from a staff member, or by a device controlled by such staff (17). However, despite such regulatory changes in Ireland, these machines remain a loophole that is potentially exploited by younger people (12,13). Donaghy *et al.* note that many youth smokers access cigarettes through what has been termed 'social sources' (18). This research aimed to explore factors influencing individuals' willingness to purchase a cigarette vending machine token for adolescents across a range of ages from 13 to 19.

METHODS

This study employed a cross-sectional survey design to assess attitudes toward assisting youth access to cigarette vending machines among adults in Ireland. Ethical approval was granted by the Research Ethics Committee of the Technological University of the Shannon (TUS). An opportunistic sample of students and staff from a provincial Technological University in Ireland was invited to participate in this research. Technological universities in Ireland offer a comprehensive range of courses, from craft apprenticeships up to PhD level. As such, they enrol more diverse cohorts of students than traditional universities (19). Data was collected via an online survey using Microsoft Forms. The data collected was analysed using descriptive and analytical statistics in R software. In addition to asking demographic and smoking status questions, the survey also inquired about participants' willingness to purchase tokens for cigarette vending machines for individuals aged 13, 15, 17, and 19 years old. A logistic regression was conducted to identify predictors of willingness to purchase a cigarette vending machine token for a 17-year-old. Independent variables included age, gender, smoking status, and parental status. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to assess the strength and precision of associations.

RESULTS

Data was collected from 599 participants aged 18 to 81. Of these 372 (62.1%) were female, 205 (34.2%) were male, and 22 (3.7%) identified as non-binary, other, or declined to answer. The average age was 31.1 (SD = 12.7). 168 (28.3%) of respondents were current smokers, smoking daily or less than daily.

As can be seen from Table 1, 70 (12.1%) of respondents stated that they would purchase a cigarette vending machine token for someone aged 17, with another 50 (8.6%) responding that they were unsure. Although this figure declines dramatically for 15 and 13-year-olds, a small number of individuals report that they will even purchase a token for a 13-year-old.

A logistic regression model was used to examine factors influencing respondents' willingness to purchase a cigarette vending token for a 17-year-old. The model revealed that age difference and smoking status were statistically significant predictors. As the age gap between the respondent and a 17-year-old increased, the odds of agreeing to purchase a token decreased (OR = 0.88, 95% CI: 0.83–0.93, $p < 0.001$). Both current or occasional smokers (OR = 4.51, 95% CI: 2.43–8.38, $p < 0.001$) and

Table 1. Age-Based Responses to Cigarette Vending Machine Tokens

Age of Token Recipient	Response	Cigarette Vending Machine Token
19 years of age	Yes	234 (39.1%)
	Don't Know	47 (7.8%)
	No	305 (50.9%)
17 years of age	Yes	70 (11.7%)
	Don't Know	50 (8.3%)
	No	459 (76.6%)
15 years of age	Yes	9 (1.5%)
	Don't Know	20 (3.3%)
	No	551 (92.0%)
13 years of age	Yes	3 (0.5%)
	Don't Know	4 (0.7%)
	No	577 (96.3%)

former smokers (OR = 4.24, 95% CI: 1.95–9.21, p < 0.001) were significantly more likely to agree compared to those who had never smoked. In contrast, gender and parental status were not significantly associated with willingness to assist.

DISCUSSION

From a Public Health perspective, it is disconcerting that more than 12% of respondents reported being willing to purchase a cigarette vending machine token for an underage smoker. This research helps confirm the probability of such vending machines continuing to be a conduit for underage smokers to access cigarettes, either directly themselves or via a proxy, as explored in this research. This is an important issue as cigarette vending machines, although largely banned in many European countries, are common elsewhere. Tackling youth smoking is crucial given the development of nicotine addiction and the difficulty many people face in quitting smoking.

This study highlights the role of age and smoking experience in shaping attitudes toward underage cigarette access. Younger individuals and those with current or past smoking habits were more inclined to facilitate access via vending machines. The absence of significant associations with gender or parental status suggests that personal smoking history may be a stronger influence than social role. These findings raise concerns about how token-based systems may reduce perceived accountability and also support the forthcoming prohibition of cigarette vending machines in Ireland, in line with public health goals.

Globally, many countries have implemented outright bans on cigarette vending machines (12,13). Yet, Ireland still hosts over 4,600 such machines (20), despite their ability to circumnavigate some elements of tobacco control legislation (21). As noted above, current Irish regulations require token-based rather than cash-based purchases. The purchase of a token rather than the direct purchase of cigarettes for youth smokers may provide moral or psychological distance between buyers and the health risks involved (22,23). The legal age to purchase cigarettes in Ireland is currently 18, although it is set to rise to 21 in 2028 (24, 25).

The Irish government has repeatedly announced intentions to ban cigarette vending machines, with news reports on this issue dating back almost a decade (26,27). However, this legislation is expected to take effect in autumn 2025. Section 26 of the Public Health (Tobacco Products and Nicotine Inhaling Products) Act 2023 establishes that it is an offence to sell a nicotine inhaling product by means of self-service (28). The commencement order has been issued for this specific section, Article 2(a) of the *Public Health (Tobacco Products and Nicotine Inhaling Products) Act 2023 (Commencement) (No. 2) Order 2024 (S.I. No. 269 of 2024)* sets the commencement date as 29 September 2025. However, there is a long history of lax regulatory enforcement of public health-related legislation in Ireland (29-35). The threat of a potential U-turn by the Irish Government is of particular concern given both the recent deferment of forthcoming alcohol warning label legislation (36) and the dramatic reversal of pioneering tobacco control legislation in Aotearoa / New Zealand (37,38).

AUTHOR CONTRIBUTIONS

FH and JMS conceptualised the study and were responsible for data collection. AA performed the analysis and wrote the initial draft. All authors contributed to the draft, its refinement and revisions. All authors reviewed and approved the final text.

COMPETING INTERESTS

The authors declare no competing interests

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Mpox Virus: Insights into Pathophysiology, Prevention, and Public Health Significance

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SUMMARY

This review paper details a comprehensive overview of Mpox virus, focusing on its epidemiology, etiological pathways of disease transmission, and pathophysiology of disease. Even though previously confined to Central and West Africa, Mpox has emerged globally, highlighting its potential for widespread human-to-human transmission. The disease's clinical presentation, viral mechanism, and progression are explored in depth. Emphasis is placed on its public health significance, especially in the context of global outbreaks, emergency preparedness and risk among vulnerable populations. Current prevention strategies, including vaccination efforts, are discussed. The paper concludes by outlining key research gaps and future directions to improve surveillance, therapeutic development, and preparedness for potential re-emergence of the virus on a global scale.

Keywords: Respiratory system diseases, Public health epidemiology, Infection risk, Mpox, Viral disease

INTRODUCTION

Monkeypox (mpox) is a rare viral disease, belonging to the poxviridae family [1]. This virus can be contracted by humans through close contact with bodily fluids from a carrier such as saliva, mucus, or skin lesions. This disease presents symptoms such as fever, muscle pain, headache, and rash. This rash begins as a small bump that evolves into raised bumps filled with fluids. Bumps caused by mpox may manifest anywhere on the body, but usually observed on the face, hands and feet. In severe cases, this illness may lead to complications such as sepsis and pneumonia which could eventually turn fatal [1].

The first virus was isolated and identified in 1959 when monkeys shipped from Singapore to Denmark research facility fell ill [2]. However, the first mpox case was confirmed in 1970, in a child in the Democratic Republic of Congo, which was initially suspected to be a smallpox case [3]. First reported human cases in the African countries were regarded endemic, but later between 1996-1997, the human to-human transmission became severe [4].

The two clades of mpox that are identified are the Central and West African clades. The Central African clades are more virulent compared to the West African [5]. A higher morbidity, mortality viremia and continuous transmission in humans was observed to be associated with the Central African clade during the 2003 outbreak in the United States (U.S) [5].

Moreover, this Central African clade is found to be more severe and fatal (10%) compared to the West African clade, which has a fatality rate of 4% [6].

Mediators of transmissions include long term close contact with contaminated personal items, respiratory droplets, and direct contact with the rash region of the infected individual. After approximately thirty years, U.S reported a mpox case-outside the African continent-due to animal importation and travel from African countries [7]. From this point on, there have been mpox cases confirmed occasionally worldwide. In 2022, mpox outbreak spread rapidly and became an international concern, and was declared as a global, health emergency independent of travel issue [8]. During this time, the World Health Organization (WHO) suggested using "mpox" instead of "monkeypox". On 16th January 2023, WHO presented the "2022 Mpox Outbreak" reporting mpox in 110 countries, territories and areas [9].

Initially, mpox was reported to be similar to a smallpox infection but with less fatality. However, with

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years, the virus became more pathogenic and caused outbreaks with many concerns being unanswered [7].

The main objective of this research paper is to provide a comprehensive literature review of the mpox virus, and exploring into its descriptive epidemiology, etiologic pathways, disease mechanism, prevention efforts and future direction of research.

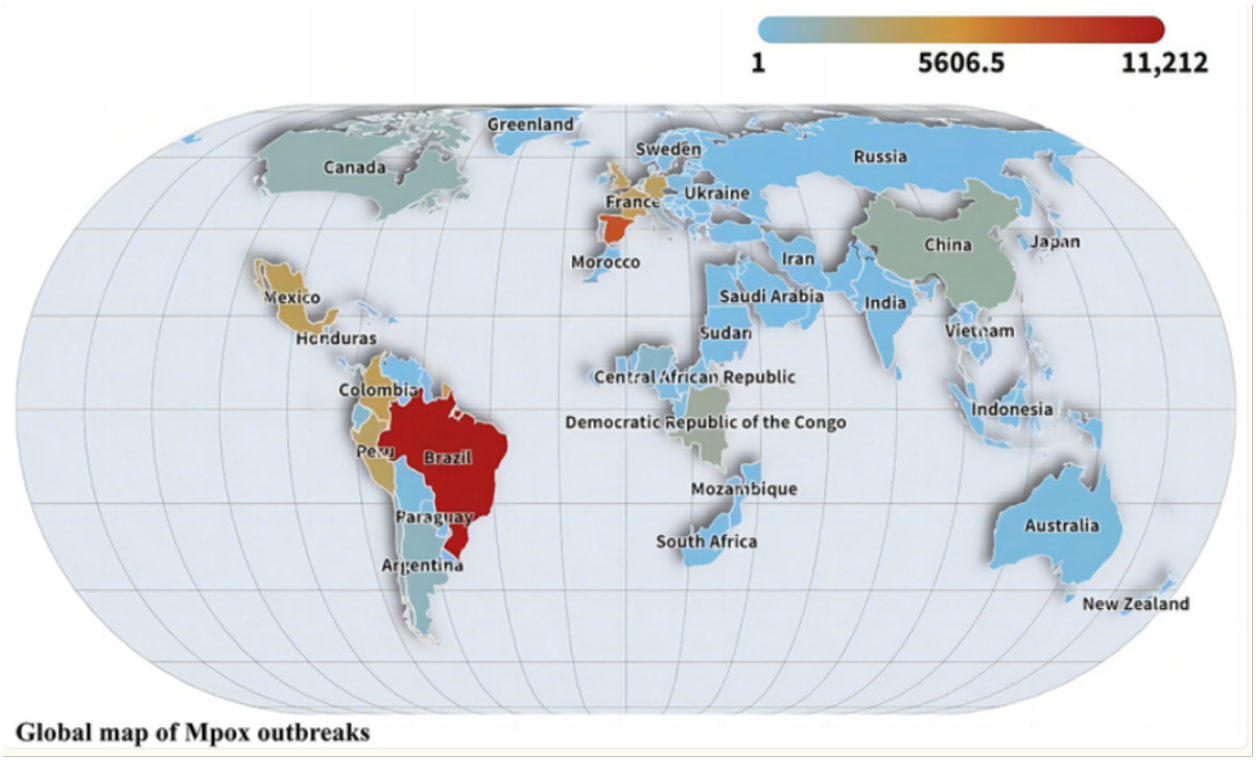
Descriptive Epidemiology

Human mpox received minimal global attention until its first outbreak that occurred in the U.S, outside of Africa in 2003 [10]. Forty-seven mpox cases were reported, with 37 confirmed, and 10 suspected. In the same year, the Republic of Congo reported the first outbreak of human mpox, where 11 patients were confirmed and probable, all of whom were 18 years or younger. Among these patients, one death was reported [11]. In 2005, between September and December, ten confirmed and nine possible patients of mpox were detected in 5 villages-Modin, Nuria, Wang Kay, Bentiu, Rubkona. From 2010 to 2018, several African countries-including the Democratic Republic of the Congo (DRC), Central African Republic (CAR), Cameroon, Liberia, Sierra Leone, and the Republic of the Congo-documented differing numbers of mpox cases. Later in 2017, Nigeria experienced a mpox outbreak, with 122 confirmed or suspected cases of human mpox reported between September 2017 and September 2018 in 17 states. Moreover, six individuals died from mpox (case fatality rate 6%) [12-14].

The Mpox outbreak has infected several individuals around the globe in 2022, which followed several sporadic cases outside of Africa, particularly United Kingdom [15], Singapore [16, 17], U.S [18]. In the UK, several cases of mpox were identified during May 2022. On epidemiologic investigation it was found that the infected individual had a recent travel history to Nigeria. The confirmed mpox cases in other countries such as Spain, Canada, and Portugal were 7, 13, 14 cases, respectively. On 18th May the U.S reported its first mpox case of 2022. Sweden and Belgium confirmed their first cases in 2022 [11]. On May 20th two patients were diagnosed with mpox in Australia both of whom had a recent travel history to Europe [15]. The first cases were later reported in France, Germany, Netherlands and France [19]. The first cases were confirmed in Israel and Switzerland, and the patient was documented by Israeli Ministry of Health as the first Asian case [20]. Furthermore, after May 2022 large number of patients were identified in non-endemic countries worldwide and the WHO declared mpox as an international public health emergency [21]. Figure 1. describes the global mpox outbreaks, with Brazil and Morocco having the highest disease burden as of October 6, 2024 [22]. Across all six WHO regions there were 57, 995 mpox cases with laboratory diagnosis reported in more than 100 countries or regions. A total of 18 deaths in 9 countries were reported [11].

During the height of the outbreak in August 2022, the rate of mpox cases was significantly higher among

*Figure 1. Global map of mpox outbreaks**



* Source: [22]

non-Hispanic Black and Hispanic or Latino males (RR=6.9) compared to White males (RR=4.1) [23]. The mpox outbreak previously disproportionately impacted, the gay, bisexual, and other men who have sex with men (MSM), along with racial and ethnic minority communities, as they experience higher rates of infection [24].

Etiological Pathways

Within the Poxviridae family, under the choropoxvirinae subfamily, orthopoxvirus genus, with its specific species identified as the mpox virus. Under the electron microscope, the virus appears as a brick-shape which is encompassed by a lipoprotein with a linear double stranded DNA, measuring 200-250 nanometers [4, 25]. The mpox virus is a zoonotic disease that is transmitted from animals to humans. The animal reservoirs identified are monkeys, rats, squirrels, and other primates, pigs, hedgehogs, prairie dogs and mice-primarily found in the African regions where the virus is historically prevalent [4].

The primary culprit of spread of is human-to-human transmission via respiratory droplets, direct contact with rashes or infected lesions, or fomites. A recent study published an analysis that detected high concentrations of the virus in bodily fluids such as saliva, feces, urine, and semen. Additionally swabs from the oropharynx and rectum confirmed that sexual transmission plays a significant role in the spread of the illness [26]. Another study recorded that the infection could also be acquired through the consumption of undercooked meat [27], or through bites or scratched from infected animals [28]. Infected mothers can spread the infection to their newborns through vertical transmission [29, 30]. Previously, mpox was only detected when an individual was either travelling to a region affected by mpox or came in contact with an infected animal [31]. Recently, majority of reported mpox cases from outbreaks have been among bisexual and gay men. This group contributed to approximately 98% of mpox cases, where 41% were coinfected with human immunodeficiency virus (HIV) and 73% had lesions on their genital or anal regions [32].

Pathophysiology

Mpox is a transient disease for most individuals but the severity of the infection depends on various factors such as strain of infection, the immunity of an individual etc., [33]. Previous literature highlights the lifecycle of the mpox virus, the first is the virus invasion, second is the viral replication and synthesis and the final is the virus assembly, maturation and release [27].

The period of incubation among mpox cases is approximately 7 to 14 days, where symptoms are observed to be present for 14 to 21 days [34]. Accurate diagnosis becomes difficult if the incubation period is prolonged, leading to delay in seeking medical care, worsening of the infection and increased risk of the spread [35].

Fever, pain, lymphadenectasis (inguinal lymphadenectasis), and fatigue are some of the common symptoms observed [36]. Lymphadenectasis usually seen to be present in mpox virus cases and can help differentiate from other orthopoxviruses [37]. After exposure through fluids from an infected individual the virus invades surrounding tissue of broken skin. Further, it disseminates throughout the body by way of local immune cells and nearby lymph nodes. [38]. During the latent period, the mpox case is usually asymptomatic and has no lesions present. On completion of the latent period the individual enters the symptomatic period where early symptoms are experienced, these prodromal symptoms-such as fever, headache, lymphadenectasis, chills, and muscle pain-persist for about three days. As the disease progresses, a rash begins to appear on face and the head region, which is later seen to spread throughout the body. From the rash, papules arise and are seen to develop, followed by the formation of vesicles and then pustules. The lesions crust over and heal, often resulting in scarring. This period of the rash usually lasts for 2 to 4 weeks [4, 39].

Individuals with weakened immune systems are generally at a greater risk of developing severe forms and complications of mpox. Moreover, these immunocompromised populations could contribute as a vital factor in driving the evolution of the mpox virus, enabling it to better adapt to human hosts and increasing the likelihood of broader transmission [40]. Complications of mpox includes inflammation of vital organs, necrotic disease, septicemia, obstructive disease, and hemorrhagic disease. In non-epidemic regions, the case fatality rate was approximately 0.04% in the year 2022 [41].

Prevention

The treatment of mpox depending on the stage of the lifecycle of the mpox virus has proved to be beneficial [27]. Cidofovir and its derivative Brincidofovir, Ribavirin are few drugs of choices used when the virus is in the second stage. Tecovirimat is a promising drug of choice for the third stage of viral assembly and maturation [27]. Even though immunopathology caused by the mpox virus can result in negative clinical outcomes, immunotherapy offers potential to lessen the severity of the illness. Polymerase Chain Reaction (PCR) is the laboratory method used to diagnose mpox by detecting the virus, even though alternative techniques such as immunological assays and virus isolation through cell culture exist, they are less commonly used [42].

Due to the immune cross-protection shared among orthopoxviruses, smallpox vaccines based on the vaccinia virus have been recommended for use during the current mpox outbreak [18]. Rimoin et al. reported a significant rise in monkeypox cases in

the Democratic Republic of Congo occurring three decades after the end of smallpox vaccination efforts [43]. Literature highlights the increased protection from mpox infection among individuals that were vaccinated against smallpox vaccine even though the vaccine was administered more than 25 years ago. The 2013 U.S mpox outbreak showed that the smallpox vaccination appears to demonstrate a protective effect against the West African clade by offering cross protective immunity [11]. In order to reduce the risk of contracting mpox, Center for Disease Control and Prevention (CDC) recommends avoiding close contact with infected individuals, especially skin contact with individuals that developed rashes and vesicles. Items and surfaces that have been used or touched by an infected person should be avoided or cleaned thoroughly. Additionally, the use of alcohol-based hand sanitizers before eating or touching their face, and washing their hands regularly, especially after using the restroom should be practiced [44].

Approximately 85% protection against mpox virus is offered by the first generation live vaccinia vaccines, as they trigger cross-reactive antibodies that help in responding to various orthopoxviruses [11]. On evaluating the benefit of the aerosolized mpox virus in cynomolgus macaques, studies showed that a single dose of the ACAM2000 vaccine offered full protection [45]. The JYNNEOS in the U.S, the IMAMUNE in Canada, and the IMVANEX in the European Union received approval in the year 2019 for use among individuals aged 18 years and older, who are susceptible to smallpox or mpox, in order to prevent both the diseases. Amid the ongoing global disease burden of mpox, pharmaceutical companies rush to develop mpox-specific vaccines similar to the COVID-19 response. Moderna has revealed that they have started exploring the possibility of creating an mRNA vaccine for mpox due to increasing vaccination demand, although no additional details have been provided so far [11].

Public health implications

One of the most effective strategies to prevent the spread of mpox infection is vaccination. Additionally, another crucial component is contact tracing and early detection of infected individuals to prevent further transmission. These screening efforts include monitoring individuals with symptoms consistent with mpox. Public health authorities, or epidemiologists may encourage targeted screening in high-risk populations such as close contacts of confirmed cases or areas that are experiencing an outbreak. Local, state and national health departments should increase surveillance efforts [46] that include syndromic surveillance improved case reporting, laboratory surveillance and rapid data analysis. These analyzed findings should be interpreted and communicated to the public in a timely manner to increase disease awareness and prevention efforts. Furthermore, One Health approach strategies that incorporate animal health monitoring can offer early indicators of potential spillover events from wildlife to humans [47]. Studies show that communication across multiple platforms such as media and news channels successfully engage diverse audiences and improve awareness and prevention strategies [42]. Increasing capacity is essential for improving readiness and response to outbreaks. It is important that healthcare professionals are trained in the correct application and removal of personal protective equipment (PPE) to reduce the likelihood of infection during patient interactions [48]. Developing and low- to middle-income countries (LMICs) should adopt to health policies that allow the emergent utilization of mpox vaccines, especially since conducting large-scale efficacy trials in these populations may not be feasible [49].

Future Research

Although mpox has become more prevalent globally, many still view it as a newly emerging infection. Therefore, continued investment in research and funding is crucial to improve public understanding and awareness, especially in areas outside of Africa where the virus is now spreading. There is currently a limited amount of research dedicated to the development of vaccines specifically targeting mpox infection.

Numerous countries have recently reported cases of mpox infection, yet there is a lack of comprehensive studies detailing the strategies used to control its spread. Publishing research on emergency preparedness is crucial, especially for developing and underdeveloped countries. Such research can inform the creation of emergency programs and training that provide these regions with the essential resources and skills needed to effectively manage and control future outbreaks.

Although progress has been made in understanding the virus and developing vaccines, significant knowledge gaps remain-particularly in areas such as viral pathogenesis, long-term immunity. Investigating the immune responses elicited by mpox infection will be vital for improving current vaccines and creating new, more targeted immunization strategies. Additionally, there is a lack of research exploring the vaccines development for immunocompromised individuals. Research should also explore the genetic determinants of virulence and host range, which could lead to the design of safer and more effective vaccine platforms.

CONCLUSION

Mpox is one of the diseases that the WHO has declared as an emergent illness worldwide, apart from COVID-19 and polio. Initially confined to Africa, mpox has now emerged as a global public health issue with cases reported internationally as well as found in communities within individual countries. The rise in mpox infections, driven by a combination of natural

and human factors, highlights an urgent need for further research. The comprehension of immune response and the mechanism of mpox virus is essential as it could offer valuable strategies and insights for advancing the development of vaccine, which is a dire need at the moment. Moreover, further research is urgently needed to develop vaccine strategies for individuals with severe immunosuppression, particularly those that do not rely on CD4-positive T cell assistance. This research is crucial to address existing gaps in vaccine development, especially given that the severe complications of mpox can be fatal.

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Systematic review of treatment options for gastric cancer and future therapeutic perspectives

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ABSTRACT

Background: Gastric cancer is the fourth most prevalent type of cancer and the second leading cause of cancer-related mortality worldwide, with an annual global incidence of 1 million cases and 700,000 deaths. Treatment modalities include surgery, chemotherapy, radiation therapy, and novel biological agents such as immune checkpoint inhibitors. The aim of the study is to summarise the existing literature on current treatment modalities and explore novel and emerging approaches to provide a detailed understanding of future advances in gastric cancer management.
Methods: A systematic review was conducted from September 2022 to May 2024 using the online databases PubMed, Scopus, and Google Scholar. The risk of bias assessment was carried out using the Newcastle-Ottawa Scale.
Results: The final review comprised 68 records. The analysis revealed that laparoscopic gastrectomy and other minimally invasive surgical approaches have yielded promising outcomes, either as standalone procedures or in combination with neoadjuvant and adjuvant chemotherapy regimens. The management of gastric cancer has been transformed by Human Epidermal Growth Factor Receptor 2-targeting agents, checkpoint inhibitors and other immunotherapies, with trastuzumab providing significant benefits when combined with chemotherapy.
Conclusion: Larger prospective or randomized controlled trials should be conducted, incorporating neoadjuvant chemotherapy regimens, targeted agents, or other innovative approaches, to confirm current research findings and enhance the efficacy and safety of various therapeutic strategies. A thorough evaluation of existing treatments and novel therapeutic interventions is imperative to guide future research initiatives, formulate effective patient care strategies, and inform policy makers.

Keywords: gastric cancer; Helicobacter pylori; monoclonal antibodies; immunotherapy; systematic review.

INTRODUCTION

Gastric cancer (GC) is an aggressive disease and a major global health problem. Overall incidence and mortality from GC have decreased significantly in recent years. The global incidence of late-onset GC fell from 59.53 per 100,000 in 1990 to 41.26 in 2019, with an average annual percentage change (AAPC) of –1.23 (95% confidence interval (CI) -1.39 to-1.06; p<0.001), while the incidence of young-onset GC (diagnosed in individuals under the age of 40) decreased from 2.20 per 100,000 in 1990

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to 1.65 in 2019, with an AAPC of –0.95 (95% CI –1.25 to-0.65; p<0.001). The mortality rates for both young- and late-onset GC decreased during this timeframe with an AAPCs of –1.82 for young-onset (95% CI-2.15 to-1.56; p<0.001) and –1.69 for late-onset GC (95% CI –1.79 to-1.59; p<0.001) [1]. Despite these improvements, GC is still the fourth most common cancer and the second leading cause of cancer-related deaths worldwide. It is still diagnosed yearly in about 1 million people and is responsible for more than 700,000 deaths, accounting for 8% of all cancer cases and 9.7% of all cancer deaths [2].

Men are two to three times more likely to develop GC than women. The number of cases varies greatly by geographical area. The regions most likely to develop GC are Central and South America, Eastern Europe, and East Asia, while Australia and New Zealand, South Asia, North and East Africa, and North America are the low-risk regions. The incidence of GC increases steadily with age, with the average age of diagnosis being 70 years. However, about 10% of GC is found in people aged 45 years or younger [3]. Although the incidence is decreasing due to improved diet, food preservation methods, better prevention strategies, and earlier detection and treatment, the disease is associated with a poor prognosis [4].

Despite the marked decrease in distal intestinal-type GC, there has been an increase in proximal diffuse gastric cardia-type adenocarcinoma in Western countries. *Helicobacter pylori* (*H. pylori*) infection and dietary habits are the major risk factors associated with distal GC. *H. pylori* is the most important etiologic factor for GC and accounts for approximately 89% of cases worldwide. The prevalence of *H. pylori* infection is higher in Central and South America, as well as in parts of Asia and Eastern Europe, compared to North America, Australia, and Western Europe [5]. Its eradication has been linked to a decrease in the incidence of GC, but the rise in antibiotic resistance to commonly used treatments like metronidazole and clarithromycin is driving the failure of eradication efforts. Prophylactic vaccination against *H. pylori* shows promise as a potential option, but a commercial vaccine is not yet available on the market [6]. In contrast, gastro-esophageal reflux disease and obesity are key factors contributing to proximal GC [7].

The biological differences in the tumours between Eastern and Western countries make it difficult to determine the standard of care based on international trials [8]. The introduction of early detection programs and new surgical techniques has led to improved survival rates for patients with localized disease, but the average 5-year survival rate for patients with advanced GC is still only 3.1% [9]. This extremely poor outcome highlights the need for better comprehensive surgical treatment of advanced GC and to promote the potential development of new therapeutic approaches. Surgery, chemotherapy, and radiation therapy have been the top treatment modalities for upper gastrointestinal malignancies for the last three decades, with the only potential cure being surgical resection. However, this has changed with the development of immune checkpoint inhibitors (ICIs), which move the protein model to a new level by providing patients with unique and long-lasting periods of improvement without surgery [10]. Nevertheless, challenges include the identification of suitable patient populations, the overcoming of resistance mechanisms, and the addressing of inter-patient variability. Meanwhile, molecular profiling and biomarker discoveries are the driving force behind the new era of precision medicine, offering the chance to increase the efficacy of therapy while minimizing side effects.

This systematic review aims to synthesize the existing literature on current treatment modalities and explore novel and emerging approaches to provide a detailed understanding of future advances in GC management.

METHODS

The present systematic review follows the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines [11]. The protocol of the study is available on Zenodo [12].

Search strategy

An extensive search was conducted in September 2022 and updated in May 2024 using three electronic databases: PubMed, Scopus, and Google Scholar. The following search string was created with the most appropriate MeSH terms and Boolean operators and adapted for each of the databases: *(Gastric OR Stomach) AND (Cancer OR Neoplasm OR Tumour* OR Adenocarcinoma) AND (treatment OR therapy OR antineoplastic OR neoadjuvant OR immunotherapy OR chemotherapy OR molecular targeted therapy)*. In addition, the references from identified systematic reviews were screened for eligible articles. Studies were eligible for inclusion in the systematic review if they met the following criteria: a) original research; b) published in English and French languages in the last decade, i.e., between 2013 and 2023 (updated to May 2024); c) studies reporting treatment options for any type of GC; d) studies including future therapeutic perspectives or directions; and e) retrospective and prospective observational studies, such as cohort, case-control, and cross-sectional studies with more than 30 GC cases. Records were excluded if GC data were merged with those from different types of cancers of the digestive tract or with cancers originating from other systems (i.e., gastroesophageal, gastrointestinal, neuroendocrine cancers).

Study selection

The title and abstracts of the retrieved records were downloaded and imported into Rayyan, enabling the removal of duplicate records [13]. The remaining

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records were then screened by four reviewers working in pairs, with any disagreements resolved through consensus-based discussions.

The full-text screening followed the same inclusion criteria as the title and abstract phase.

Data extraction

An extraction form was created, based on the Joanna Briggs Institute (JBI) Manual for Evidence Synthesis [14], to collect data on:

- study characteristics (first author, publication year, country of the research);
- study methods (study design, patients' inclusion criteria, duration of the study);
- sample characteristics (size, age, gender, ethnicity/race, clinical stage of the GC according to the tumour, node, and metastasis classification, presence of metastasis, treatment modality and lines, adverse effects);
- primary and secondary outcomes, future directions or recommendations provided by the authors.

Each reviewer conducted an independent extraction that was checked by a second reviewer for accuracy.

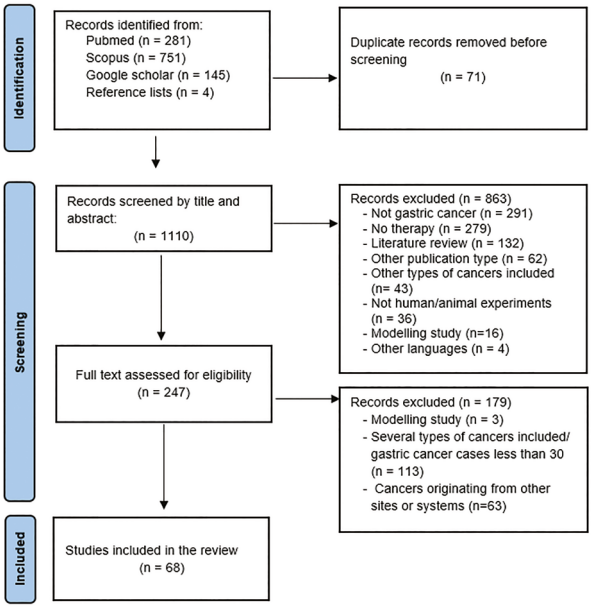
Quality assessment

The risk of bias assessment was carried out after the data extraction phase using the Newcastle-Ottawa Scale (NOS) for cohort, case-control [15] and cross-sectional studies [16]. The tool for cohort and case-control studies consisted of three domains: selection (four points), comparability (two points), and outcome (three points). The adapted version for cross-sectional studies differed from the original tool in the maximum achievable score in the selection domain (five points). The results of the assessment are also presented according to the Agency for Healthcare Research and Quality (AHRQ) standards. The thresholds for converting the NOS to AHRQ standards (good, fair, and poor quality studies) [17] are reported in Table 1.

RESULTS

Sixty-eight studies, published between 2013 and 2024, were included in the systematic review (Figure 1).

Figure 1. Flow chart of the selection process indicating the number of selected articles for each step of the systematic review on gastric cancer and future perspectives



The main characteristics of the studies are depicted in Table 2.

Table 1. Threshold values for converting the NOS to AHRQ standards of the included studies

Cohort and case-control studies	Points in Selection Domain	Points in Comparability Domain	Points in Outcome Domain
Good	≥3	≥1	≥2
Fair	2	≥1	≥2
Poor	0-1	0	0–1
Cross-sectional studies*			
Good	≥4	≥1	≥2
Fair	≥2	≥1	≥2
Poor	0–1	0	0–1

NOS, Newcastle-Ottawa Scale; AHRQ, Healthcare Research and Quality Standards; *Based on AHRQ Methodology Checklist for cross-sectional studies [18].

Table 2. Characteristics of the studies on gastric cancer and future therapeutic perspectives

First Author, year	Country	Study design	Inclusion Criteria	Study duration (years)	Number of patients (N)	Age (mean ± SD or range)	Ethnicity/ Race
Ahn, 2014 [53]	ROK	Clinical trial	Untreated, pathologically proven advanced GC with measurable lymph node metastases, ECOG 0–1, age 18–75, ASA I–II, adequate organ function, no prior chemotherapy/radiotherapy	3.5	140 (neoadjuvant chemotherapy = 48; surgery alone = 92)	Neoadjuvant: 53.8 ± 8.9; Surgery alone: 58.9 ± 11.2	NR
Ali, 2023 [56]	Pakistan	Retrospective cohort	Operable GC with lymphadenectomy; received perioperative or adjuvant chemotherapy	6	108	27–80 (range)	NR
Bao, 2017 [32]	China	Observational study	GC meeting surgical indications, R0 resection, ≥1 cycle adjuvant chemotherapy after radical gastrectomy	7	286 (Laparoscopic = 157; Open = 129)	Laparoscopic: 61 (42–70); Open: 59 (40–69)	NR
Beeharry, 2019 [24]	China	Case-control	Age 18–76, T ≥ 3 by staging, KPS > 50, adequate laboratory values, no major comorbidities; randomized to D2 resection ±HIPEC	0.5	80 (HIPEC = 40, Control = 40)	HIPEC: 59 ± 10; Control: 58 ± 10	NR
Chen, 2019 [22]	China	Case-control	Poor performance status (2 or 3), advanced GC, ≥2 prior lines of chemotherapy, declined additional chemotherapy, consenting to Apatinib + BSC vs. BSC alone	2.2	61 (apatinib group = 20; control = 41)	41–79 (range)	NR
Chen, 2021 [29]	Japan	Prospective cohort	Unresectable advanced/recurrent GC, receiving ramucirumab for the first time in routine clinical practice	3.6	609	21–94 (range)	NR
Cho, 2020 [51]	ROK	Cohort	Pathologically proven advanced GC with acute bleeding requiring transarterial embolization	10	58	62.5 ± 12.8	NR
Choi, 2018 [59]	ROK	Cohort	Histologically confirmed recurrent/metastatic GC, received ≥1 line of palliative chemotherapy	11	682	81.8% < 70 years (exact mean NR)	NR
Choi, 2019 [45]	ROK	Cohort	Underwent EMR or ESD for premalignant lesions or early GC; length of stay ≤2 days	11.3	914	63.4 (mean)	NR
Cordova-Delgado, 2021 [30]	Chile	Case-control	Histologically confirmed GC; ≥2 cycles of fluoropyrimidine ± platinum chemotherapy; adequate organ function; age >18; available biological sample	12.9	93 (cases=32; controls=61)	>18 (range not specified)	Latin
Deftereos, 2021 [31]	Australia	Prospective observational	Age ≥18, inpatient, curative gastrointestinal surgery (gastrectomy/esophagectomy/pancreatectomy), SGA by dietitian within 7 days, adequate communication	0.8	50 (GC subset only)	67 ± 10	NR
Dong, 2016 [35]	China	Case-control	Age 30–70, Borrmann type II/III, no distant metastases, T2–T3; Groups: FOLFOX6, SOX, XELOX vs. Surgery alone	3	603 (control = 141, FOLFOX6 = 157, SOX = 160, XELOX = 145)	Median 54	NR

(*continued*)

Table 2. Characteristics of the studies on gastric cancer and future therapeutic perspectives (continued)

First Author, year	Country	Study design	Inclusion Criteria	Study duration (years)	Number of patients (N)	Age (mean ± SD or range)	Ethnicity/ Race
Dong, 2018 [60]	China	Case-control	Advanced or metastatic GC or locally advanced GC not suited for surgery, no history of other malignancies	9.8	177	20–76 (range)	NR
Gamboa-Hoil, 2020 [50]	Mexico	Cohort	GC patients (T2/T3) undergoing surgery	4	70	43–86 (range)	NR
Garbarino, 2020 [33]	Italy	Retrospective observational	Excluding cT1, cT4b, metastatic, or neoadjuvant chemotherapy; laparoscopic distal gastrectomy vs. open in a Western center	5	123 (laparoscopic = 60, Open = 63)	Lap: 72.2 ± 9.9; Open: 72.1 ± 10.1	NR
Guo, 2023 [25]	China and USA	Cohort	Primary GC stage II/III, underwent gastrectomy	13	1,636	19–98 (range)	NR
Han, 2024 [68]	China	Cohort	Diagnosed GC, received immune checkpoint inhibitors (± chemotherapy)	3	584	IQR 46–69	NR
Hao, 2024 [70]	China	Observational study	Advanced GC on immunotherapy (Dec 2017–Apr 2022)	4.3	402 (immune-related AEs = 191; non-immune AEs = 211	Mean ~63 (both groups)	NR
He, 2024 [37]	China	Observational study	HER2+ advanced GC treated with trastuzumab (2011–2019)	8	207	Training: 60.8 ± 10.7; Internal: 60.0 ± 12.8; External: 63.8 ± 10.5; Prospective: 67.0 ± 19.5	NR
Hernanz, 2019 [23]	Spain	Retrospective cohort	Underwent esophagogastroduodenoscopy, histologically proven GC, diagnosed in participating centers	8	1,289	74.1 ± 11.2	NR
Higuchi, 2013 [46]	Japan	Observational study	Early gastric tubular/papillary adenocarcinoma with ulcer scar ≤3 cm intramucosal, no distant LN, double-endoscope ESD performed	3.7	57 (double-endoscope = 30; control = 27)	Double: 67 (51–87); Control: 69 (43–82)	NR
Hsieh, 2016 [74]	Taiwan	Retrospective cohort	Age ≥18, metastatic GC, data on NLR/mGPS/PG-SGA within 1 week pre-chemotherapy, ≥1 cycle of chemotherapy for metastatic GC	7	256	26–85 (range)	NR
Huang (W), 2023 [69]	China	Cohort	Histologically confirmed GC, standard CT within 4 weeks before immunotherapy	5.3	294	IQR 48–66	NR
Huang (K), 2023 [85]	China	Retrospective cohort	High-grade dysplasia or early GC resected by ESD	7	286	62.5 ± 9.3	NR
Jeong, 2015 [75]	ROK	Retrospective cohort	Patients undergoing gastrectomy for GC	3	2,107	61.2 ± 12.0	NR
Kaito, 2017 [40]	Japan	Cohort	GC (II–III) undergoing distal or total gastrectomy + D2 lymph node dissection	4.7	148	Laparoscopic: 35–85; Open: 41–81	NR
Kalinka-Warzocha, 2015 [87]	7 European countries	Prospective observational	Adults with GC (any stage), receiving ≥3 consecutive cycles of myelosuppressive chemotherapy; febrile neutropenia risk ≥20% or <20%	2.2	163	60 ± 14	NR

(*continued*)

Table 2. Characteristics of the studies on gastric cancer and future therapeutic perspectives (continued)

First Author, year	Country	Study design	Inclusion Criteria	Study duration (years)	Number of patients (N)	Age (mean ± SD or range)	Ethnicity/ Race
Kang, 2017 [48]	ROK	Observational study	Early GC with signet ring cell histology, underwent surgery	5	789	Mean 61.98	NR
Kim, 2021 [65]	ROK	Experimental study	Excluding distant metastases, no preoperative chemotherapy/radiotherapy, analyzing MET pathway and outcomes	1.9	272	135 <60 yrs, remainder ≥60 yrs	NR
Kim, 2020 [52]	ROK	Cohort	Unresectable GC with obstruction at EGJ or pylorus (e.g., nausea, vomiting, dysphagia)	10	118	EGJ group: 67.7 ± 9.97; Pylorus: 64.6 ± 11.81	NR
Kim, 2019 [80]	ROK	Cohort	Age >20, unresectable/metastatic/recurrent GC, ECOG 0–2, no prior palliative chemotherapy, estimated survival >3 months	2.3	527	25–86 (range)	NR
Kim, 2018 [36]	ROK	Observational study	Stage II–III GC, post-D2 gastrectomy with R0 resection, no preoperative chemotherapy/radiotherapy, age 20–75, ≥25 LN examined, no synchronous/metachronous cancers, received either S-1 or XELOX adjuvant within 8 weeks	1.8	1,774 (pre-PSM = 1,088; post-PSM = 686)	Pre-PSM: S-1 ~61.4±11.7 vs. XELOX ~56.4±10.6; Post-PSM: S-1 ~59.1±12.0 vs. XELOX ~57.5±10.8	NR
Kim, 2016 [77]	ROK	Observational study	Advanced or early GC with Helicobacter pylori (+), suitable for subtotal gastrectomy, no preoperative chemotherapy, provided informed consent	2.8	169 (treatment = 87; placebo = 82)	Treatment: 58 (48–65); Placebo: 56 (48–64)	NR
Li (J), 2018 [43]	China	Retrospective observational	GC with synchronous liver metastases, comparing minimally invasive surgery vs. open approach	10.5	53 (minimally invasive surgery=11, Open=42)	MIS: 58.9 ± 3.4; Open: 56.8 ± 1.6	NR
Li (Q), 2018 [66]	China	Prospective observational	Inoperable, HER2+ advanced GC, receiving first-line palliative chemotherapy + trastuzumab, measurable lesions, ECOG PS 0–2, LVEF >50%, adequate organ function	5	107	<65 yrs = 56; ≥65 yrs = 51	NR
Li, 2020 [67]	China	Prospective cohort	HER2+ advanced/metastatic GC or EGJ cancer, 6 cycles of trastuzumab-based first-line therapy, then maintenance strategies	5.5	78	Mean 64	NR
Liu, 2015 [81]	China	Observational study	GC patients operated on Jan 2008–Dec 2013, comparing those who received IIC vs. no IIC	6	845 (IIC = 356; Control = 489)	IIC group: 56 ± 11; Control: 56 ± 12	NR
Martinez-Lago, 2015 [63]	Spain	Retrospective cohort	Histologically proven advanced GC/GEJ, curative resection with negative margins, no preoperative therapy	7	55	40–81 (range)	NR
Murat Sedef, 2019 [62]	Turkey	Retrospective cohort	Metastatic GC not treated with trastuzumab	10	516	25–85 (range)	NR
Mokdad, 2018 [26]	USA	Retrospective cohort	Gastric adenocarcinoma (all stages)	8	89,098	18 to ≥75	White, Black, Asian, Hispanic, other

(*continued*)

Table 2. Characteristics of the studies on gastric cancer and future therapeutic perspectives (continued)

First Author, year	Country	Study design	Inclusion Criteria	Study duration (years)	Number of patients (N)	Age (mean ± SD or range)	Ethnicity/ Race
Narita, 2023 [71]	Japan	Cohort	Nivolumab-refractory or intolerant advanced GC, ECOG 0–2, scheduled for subsequent cytotoxic chemotherapy, measurable lesions	2.8	199	29–87 (range)	NR
Noh, 2018 [79]	ROK	Nested case-control	Stage I GC, pathologically confirmed, bone mineral density measured just before gastrectomy or endoscopic treatment	6	49	56.5 ± 10.8	NR
Nomura, 2019 [49]	Japan	Retrospective case-control	ESD in remnant stomach vs. intact stomach, Jan 2005–Sep 2017; includes post-gastrectomy (distal, proximal, or pylorus-preserving)	12.7	3,375 (remnant=138; intact=3,237)	Remnant: 71.2 ± 7.3; Intact: 69.6 ± 9.6	NR
Oh, 2021 [57]	ROK	Retrospective observational	GC with curative resection, adjuvant S-1 vs. XELOX	7.5	1,461	<60 or ≥60	NR
Olmi, 2020 [42]	Italy	Retrospective observational	Patients with GC from Jan 2010–Jun 2018, laparoscopic approach with D2 LN dissection & omentum preservation	8.5	138	Mean 70.7 ± 10.1	NR
Oyama, 2013 [38]	Japan	Prospective observational	High or moderate emetic-risk chemotherapy-naïve adults, planned for cisplatin + S-1	NR	53	50–81 (range)	Japanese
Oyama, 2016 [39]	Japan	Observational study	Age ≥20, ECOG PS 0–2, receiving S-1 + cisplatin chemotherapy for GC	1.7	72	Median 65 (range 50–81); 34 <65, 38 ≥66	NR
Petrioli, 2020 [55]	Italy	Prospective observational	Clinical T3–T4 non-metastatic GC, Jan 2010–Dec 2017, comparing NAC with DOC vs. EOF	8	63 (DOC=34, EOF=29)	DOC median=67; EOF median=63	NR
Pyo, 2016 [19]	ROK	Prospective observational	Age >20, newly diagnosed early GC meeting endoscopic resection criteria, no prior GC treatment, curative intent	11	2,563 (ESD=1,290; surgery=1,273)	ESD median ~61; Surgery median ~59	NR
Qiu, 2023 [72]	China	Retrospective cohort	Age ≥18, pathologically confirmed GC, ECOG 0–2, ≥1 measurable lesion (RECIST 1.0), adequate organ function, receiving apatinib second line or beyond	5	92	Mean 62.9 ± 8.7 (range 30–82)	NR
Qiu, 2014 [21]	China	Observational study	Advanced GC, completed 6 cycles of first-line XELOX without progression, developed ≥grade 2 neuropathy, ≥1 measurable lesion, life expectancy ≥3 months	1.9	286 (study group=64; control=222)	Study group: 24–74; Control: 19–82	NR
Rausei, 2015 [54]	Italy	Prospective observational	No distant metastases at laparoscopy, cT ≥ 3 GC (Jan 2010–Dec 2013), comparing NAC + surgery vs. surgery alone	4	71 (NAC + surgery=10; surgery alone=61)	NAC + surgery: mean 66.2; Surgery: mean 72	NR
Saito, 2021 [27]	Japan	Cohort	Unresectable or recurrent GC with peritoneal metastases, age >20, ECOG 0–2, adequate organ function, no other distant metastases (except ovary)	3.2	44	37–77 (range)	NR

(*continued*)

Table 2. Characteristics of the studies on gastric cancer and future therapeutic perspectives (continued)

First Author, year	Country	Study design	Inclusion Criteria	Study duration (years)	Number of patients (N)	Age (mean ± SD or range)	Ethnicity/ Race
Sarriugarte, 2018 [41]	Luxembourg and Spain	Cohort	GC cT1–4 N0–3 M0 located in antrum/body; planned laparoscopic curative R0 gastrectomy	4	67	37–85 (range)	NR
Sato, 2020 [78]	Japan	Observational study	cT2 or deeper GC by endoscopy or CT; no Linitis plastica, no para-aortic LN, no stage IV, no prior staging laparoscopy	NR	1,232–1,322 (approx.)	~69 (29–92) for CE alone, similar in other subsets	Asian (Japanese)
Shi, 2021 [28]	China	Cohort	Age 18–75, metastatic GC with peritoneal metastases, measurable lesion, ECOG 0–1, no prior chemotherapy/radiotherapy/targeted/immunotherapy	2	30	29–74 (range)	NR
Shin, 2024 [76]	Korea	Retrospective cohort	Early papillary GC without LN metastasis, underwent ESD	8	176	71.9 ± 8.7	NR
Tate, 2019 [20]	Australia	Observational study	Gastric lesion >10 mm, T1 lesion (mucosal/submucosal), age ≥18	5.8	121	Overall mean ~72.0 ± 10.6 (subsets ranged 67.4–75.2)	Mixed (Asian, European, etc.)
Terashima, 2021 [83]	Japan	Cohort	Incurable advanced GC with gastric outlet obstruction, age ≥20, ECOG 0–2, adequate organ function, poor/no oral intake	NR	104	Median 68	NR
Trip, 2014 [61]	Netherlands	Observational study	Postoperative chemoradiotherapy for GC (AP-PA vs. 3D-conformal vs. IMRT)	8	87 (AP-PA=31, 3D=25, IMRT=31)	AP-PA: mean 56, 3D: 53, IMRT: 58	NR
Ushiku, 2015 [82]	Japan	Retrospective observational	GC patients who underwent gastrectomy (2005–2010)	6	790	65.2 ± 10.7	NR
Wang, 2017 [58]	China	Prospective cohort	Stage II/III GC post-gastrectomy (D2 LN dissection), comparing chemotherapy alone vs. chemotherapy + CIT	2	159	<60 or ≥60	NR
Wang, 2020 [44]	China	Cohort	Patients undergoing radical gastrectomy (stage I–IV), comparing morning vs. afternoon start	5	117	44 <65 yrs, 73 ≥65 yrs	NR
Yamamoto, 2020 [88]	Japan	Observational study (case-control)	Patients >20 yrs with gastric lesions indicated for ESD, on antithrombotics per JGES guidelines	0.9	166 (vonoprazan=50; historical control=116)	Vonoprazan: 78 (54–87); Control: 75 (59–87)	NR
Yan, 2019 [64]	China	Cohort	D2 laparoscopic radical gastrectomy for GC, ≥18 yrs, no major postoperative complications, ≥5 cycles of chemotherapy	2	108	Group A (S-1): 53.7 ± 6.8; Group B (no S-1): 54.4 ± 7.4	NR
Yang, 2015 [47]	China	Retrospective cohort	Early GC or precancerous lesions treated via ESD	9.2	83	72.7 ± 11.5	NR
Zhang, 2019 [34]	China	Retrospective cohort	GC patients <75 yrs, no severe comorbidities, normal liver/renal function, hemoglobin ≥80 g/L, no severe abdominal pain/distension, body temperature <38°C, comparing IPHP vs. none	2	1,573 (IPHP=134; non-IPHP=1,439)	IPHP: 55.5 ± 10.8; Non-IPHP: 55.4 ± 11.0	NR

(*continued*)

Table 2. Characteristics of the studies on gastric cancer and future therapeutic perspectives (continued)

First Author, year	Country	Study design	Inclusion Criteria	Study duration (years)	Number of patients (N)	Age (mean ± SD or range)	Ethnicity/ Race
Zhang, 2024 [73]	China	Observational study	Advanced GC, treated with anti-PD-1 therapy, Jan 2019–Sep 2023	4.7	158 (low AFP=138; high AFP=20)	Low AFP: <60 yrs=48 (34.8%), ≥60 yrs=90 (65.2%); High AFP: <60 yrs=6 (30%), ≥60 yrs=14 (70%)	NR

AEs, adverse events; AFP, alpha-fetoprotein; AP-PA, anterior-posterior-posterior-anterior radiation technique; ASA, American Society of Anesthesiologists; BSC, best supportive care; CIT, cellular immunotherapy; CT, computed tomography; DOC, docetaxel + oxaliplatin + capecitabine; ECOG PS, Eastern Cooperative Oncology Group performance status; EGJ, esophagogastric junction; EMR, endoscopic mucosal resection; EOF, epirubicin + oxaliplatin + 5-fluorouracil; ESD, endoscopic submucosal dissection ; FOLFOX6, oxaliplatin, leucovorin, and fluorouracil (5-FU); GC, gastric cancer; HIPEC, hyperthermic intraperitoneal chemotherapy; IIC, intraoperative intraperitoneal chemotherapy; IMRT, intensity-modulated radiation therapy; IPHP, intraperitoneal hyperthermic perfusion; IQR, interquartile range; JGES, Japan Gastroenterological Endoscopy Society; KPS, Karnofsky performance status; LAGC, nonmetastatic tumours, subserosal/serosal involvement, with or without lymph node invasion; LN, lymph nodes; LVEF, left ventricular ejection fraction; MET, mesenchymal-epithelial transition; NAC, neoadjuvant chemotherapy; NR, not reported; PD-1, programmed cell death 1; ROK, Republic of Korea; S-1, tegafur/gimeracil/oteracil; SGA, subjective global assessment; SOX, S-1 (tegafur/gimeracil/oteracil) and oxaliplatin; XELOX, capecitabine + oxaliplatin; Yrs, years

The top countries in terms of study origin are China (32.3%), the Republic of Korea (22%), and Japan (17.6%). Most of these studies adopted a prospective or retrospective cohort design (47%), while others used observational (39.7%) or case-control (10.3%) frameworks. The patients' inclusion criteria were based on the tumour stage, the patients' performance status, and their treatment history. Some studies targeted early-stage cases suitable for endoscopic resection [19, 20], whereas others investigated advanced or metastatic disease, including cases refractory to prior treatments [21,22]. Performance status and baseline organ function assessments are nearly universal, with most studies requiring an Eastern Cooperative Oncology Group (ECOG) score of 0-2. Additionally, certain studies explored unique comorbidities or risk factors [23]. The studies' duration ranged from 6 months [24] to a maximum of 13 years [25] and included a wide range of sample sizes. A study in the USA enrolled 89,098 patients with all stages of gastric adenocarcinoma [26]. In contrast, two studies had just 44 and 30 participants in Japan [27] and in China [28], respectively. Age ranges in the included studies reflect the predominance of patients in their 50s and 60s, though some cohorts span from young adults to elderly individuals aged over 90 [25, 29]. All studies included both sexes in varying proportions. When reported, ethnicity or race was often listed broadly, particularly in Asian-based studies. In contrast, a study offered a more diverse representation [26] including White, Black, Asian, Hispanic, and other racial categories across the United States. In Chile, a study evaluated a Latin cohort [30], while Australian-based studies [20, 31] reported a mix of Asian and European participants.

Treatment approaches varied extensively (Table 3). Some studies compared laparoscopic versus open gastrectomy [32, 33], while others examined the role of hyperthermic intraperitoneal chemotherapy [24, 34] or different neoadjuvant and adjuvant regimens [35, 36]. Other studies explored targeted therapies [29] and evaluated HER2-targeted regimens with trastuzumab in a Chinese cohort [37], or focused on supportive or adjunctive measures (e.g., antiemetic control in cisplatin-based chemotherapy) [38, 39] (Table 4).

Minimally invasive or local interventions

Laparoscopic gastrectomy and other minimally invasive surgical approaches have yielded promising outcomes in GC. They could reduce blood loss, diminish postoperative pain, facilitate faster recovery, and improve overall survival (OS)/disease-free survival (DFS) [32, 33]. Performing a laparoscopic gastrectomy may allow for faster initiation of adjuvant chemotherapy [40], especially when combined with D2 lymph node dissection for robust oncological outcomes and low leak risk [41]. Notably, preserving the omentum during laparoscopic surgery appeared feasible and safe for both early and advanced disease. Indeed, patients with omentum preservation had a lower incidence of relapse compared to those with omentectomy (40% vs 57%; p=0.002) [42]. Comparable long-term results were also observed, with reduced blood loss and faster oral intake [43]. However, the timing of surgery might matter: afternoon distal gastrectomies were associated with more bleeding (227.88±181.79 vs 117.93±112.01; p<0.001), slower gastrointestinal recovery, and worse OS [44]. In addition, endoscopic submucosal dissection (ESD) and endoscopic mucosal resection (EMR) are effective for early GC or premalignant lesions. Performing EMR/ESD within two

Table 3. Non-pharmacological interventions and adverse events

First Author, year	Non-pharmacological interventions	Adverse events
Ahn, 2014 [53]	Surgery alone (radical gastrectomy with D2)	Death, morbidity (intra-abdominal bleeding, fluid collection, anastomotic leak, pneumonia)
Ali, 2023 [56]	Surgery	NR
Bao, 2017 [32]	Laparoscopic gastrectomy vs open surgery	NR
Beeharry, 2019 [24]	Control group: surgery alone	NR
Chen, 2019 [22]	Supportive therapy	NR
Chen, 2021 [29]	None	NR
Cho, 2020 [51]	TAE	NR
Choi, 2018 [59]	NR	NR
Choi, 2019 [45]	EMR/ESD	NR
Cordova-Delgado, 2021 [30]	Chemotherapy ± radiotherapy ± surgery	Not fully detailed
Deftereos, 2021 [31]	Surgery (open, laparoscopic)	Malnutrition and weight loss: longer stay
Dong, 2016 [35]	Radical D2 gastrectomy (control group)	NR
Dong, 2018 [60]	Chemoradiotherapy	NR
Gamboa-Hoil, 2020 [50]	Radiotherapy (median 50.4 Gy), surgery (subtotal or total)	NR
Garbarino, 2020 [33]	Gastrectomy + D2 LN dissection (lap vs open)	Conversions from laparoscopy to open surgery (n=5)
Guo, 2023 [25]	Surgery (open or laparoscopic)	NR
Han, 2024 [68]	NR	NR
Hao, 2024 [70]	NR	NR
He, 2024 [37]	NR	NR
Hernanz, 2019 [23]	Surgery (Billroth I/II, Roux-en-Y)	NR
Higuchi, 2013 [46]	Possible surgical resection if ESD fails	NR
Hsieh, 2016 [74]	None	NR
Huang (W), 2023 [69]	NR	NR
Huang (K), 2023 [85]	ESD ± radical gastrectomy	NR
Jeong, 2015 [75]	Surgery (open or laparoscopic)	Local: ascites, GI bleeding, anastomotic leak. Systemic: pulmonary complications
Kaito, 2017 [40]	Distal or total gastrectomy (laparoscopic vs open surgery)	Anastomotic leak, pancreatic fistula, bowel obstruction, pneumonia
Kalinka-Warzocha, 2015 [87]	Surgery ± chemotherapy ± radiotherapy	NR
Kang, 2017 [48]	Subtotal/total gastrectomy + D1 + or D2 LN dissection	Not specified
Kim, 2021 [65]	NR	NR
Kim, 2020 [52]	Self-expandable metal stent in EGJ vs pylorus	Bowel perforation, stent migration, bleeding
Kim, 2019 [80]	Subtotal/total gastrectomy	NR
Kim, 2018 [36]	NR	NR
Kim, 2016 [77]	Subtotal gastrectomy	NR
Li (J), 2018 [43]	Robotic or laparoscopic resection ± RFA ± hepatectomy	NR
Li (Q), 2018 [66]	NR	NR
Li, 2020 [67]	NR	NR

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Table 3. Non-pharmacological interventions and adverse events (continued)

First Author, year	Non-pharmacological interventions	Adverse events
Liu, 2015 [81]	Gastrectomy	NR
Martinez-Lago, 2015 [63]	Gastrectomy	NR
Mokdad, 2018 [26]	Surgery	NR
Murat Sedef, 2019 [62]	Surgery	NR
Narita, 2023 [71]	NR	NR
Noh, 2018 [79]	Gastrectomy vs endoscopic treatment	NR
Nomura, 2019 [49]	Possible re-surgery if incomplete ESD	NR
Oh, 2021 [57]	Gastrectomy ± endoscopic approach	NR
Olmi, 2020 [42]	Laparoscopic D2 gastrectomy with omentum preservation	Complications, length of surgery, length of stay
Oyama, 2013[38]	None	NR
Oyama, 2016 [39]	NR	NR
Petrioli, 2020 [55]	Gastrectomy (D2 or D3 LN dissection)	NR
Pyo, 2016 [19]	Endoscopic resection (ESD or EMR) vs surgical resection	Some differences in short-term complications
Qiu, 2023 [72]	NR	NR
Qiu, 2014 [21]	None	NR
Rausei, 2015 [54]	Surgery alone vs NAC + surgery (gastrectomy ± LN dissection)	9 in surgery-only vs 0 in NAC group
Saito, 2021 [27]	Conversion gastrectomy	Post-operational leak
Sarriugarte, 2018 [41]	~95% laparoscopic gastrectomy + D2 LN, Roux-en-Y	Leak, bleeding, infection
Sato, 2020 [78]	None	NR
Shi, 2021 [28]	Conversion surgery (R0, D2 LN)	NR
Shin, 2024 [76]	EMR or ESD	NR
Tate, 2019 [20]	Surgery if incomplete prior resection	NR
Terashima, 2021 [83]	Surgical palliation (distal/total gastrectomy or EGJ)	NR
Trip, 2014 [61]	None (radiologic approaches compared)	NR
Ushiku, 2015 [82]	Gastrectomy	SSI (incisional, organ/space)
Wang, 2017 [58]	D2 gastrectomy	NR
Wang, 2020 [44]	Radical gastrectomy	NR
Yamamoto, 2020 [88]	ESD	NR
Yan, 2019 [64]	Total or distal gastrectomy	NR
Yang, 2015 [47]	Surgery if needed	NR
Zhang, 2019 [34]	Gastrectomy (total/subtotal/palliative) + chemotherapy	NR
Zhang, 2024 [73]	NR	NR

EGJ, esophagogastric junction; EMR, endoscopic mucosal resection; ESD, endoscopic submucosal dissection; GI, gastrointestinal; LN, lymph node; NAC, neoadjuvant chemotherapy; RFA, radiofrequency ablation; SSI, surgical site infection; TAE, transarterial embolization

Table 4. Pharmacological interventions, treatment lines and adverse events

First Author, year	Pharmacological interventions	Treatment lines	Adverse events
Ahn, 2014 [53]	NAC: 4 cycles mFOLFOX6 prior to surgery, 4 cycles adjuvant mFOLFOX6 post-surgery	NR	IP bleeding, morbidity, wound problems, fluid collections, anastomotic leak, thrombophlebitis
Ali, 2023 [56]	Adjuvant (CAPOX) or perioperative (ECF/FLOT)	NR	NR
Bao, 2017 [32]	Various chemotherapies: IV 5-FU + cisplatin, oral fluoropyrimidines ± S-1, etc.	NR	Grade 3/4 toxicities (cytopenia, GI)
Beeharry, 2019 [24]	HIPEC (cisplatin 50 mg/m² at 42°C × 60 min)	I	Mild AEs (neutropenia, renal toxicity, hyperbilirubinemia)
Chen, 2019 [22]	Apatinib (250–500 mg/day) + BSC	III+	Appetite decrease, fatigue, anemia (often grade ≥3)
Chen, 2021 [29]	Ramucirumab ± chemotherapy (often paclitaxel)	I–IV	Neutropenia, appetite decrease, hypertension, neuropathy (paclitaxel), ILD/pneumonitis (rare)
Cho, 2020 [51]	NR	NR	Stomach wall perforation (rare)
Choi, 2018 [59]	FOLFIRI, FOLFOX, paclitaxel/cisplatin, etc.	III+	NR
Choi, 2019 [45]	NR	NR	Bleeding (hematemesis, melena)
Cordova-Delgado, 2021 [30]	Multiple regimens: FOLFOX, CAPEOX, CF, DCFm, ECF, FLOT, etc.	I	Neuropathy (common grade 1), neutropenia (grade 3), diarrhea, nausea; DPYD SNPs linked to toxicity
Deftereos, 2021 [31]	NR	NR	NR
Dong, 2016 [35]	NAC: FOLFOX6, SOX, XELOX	I	Leukopenia/neutropenia, nausea/vomiting. FOLFOX6 had more liver dysfunction, constipation/pain vs SOX/XELOX
Dong, 2018 [60]	Various chemotherapies ± platinum ± docetaxel + radiotherapy	IV, locally advanced, recurrent	Mostly hematologic and GI (nausea, vomiting) at grade I–II; low incidence of grade III–IV
Gamboa-Hoil, 2020 [50]	Adjuvant XELOX, CAPEOX, FOLFOX, or capecitabine	NR	NR
Garbarino, 2020 [33]	NR	NR	No major difference in postoperative complications (laparascopic: 2 leaks vs open surgery: 4 canalization delays)
Guo, 2023 [25]	Adjuvant: S-1 alone or combos (SOX, XELOX, FOLFOX)	NR	NR
Han, 2024 [68]	ICIs ± chemotherapy	I+	NR
Hao, 2024 [70]	PD-1 inhibitors	I, II+	Skin rash, nail abnormalities, diarrhea
He, 2024 [37]	Anti-HER2 therapy (trastuzumab ± chemotherapy)	I, II, III+	NR
Hernanz, 2019 [23]	Chemotherapy (adjuvant, neoadjuvant, palliative), PPI therapy	I–IV	NR (not specifically detailed)
Higuchi, 2013 [46]	NR	NR	Delayed hemorrhage, nausea/vomiting, perforation, pneumonia, delirium (mostly grade 1–2, low incidence)
Hsieh, 2016 [74]	Fluoropyrimidine ± platinum (capecitabine+oxaliplatin, S-1, etc.)	I	NR
Huang (W), 2023 [69]	Anti-PD-1/PD-L1 ICIs ± chemotherapy	I, II, III+	NR

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Table 4. Pharmacological interventions, treatment lines and adverse events (continued)

First Author, year	Pharmacological interventions	Treatment lines	Adverse events
Huang (K), 2023 [85]	Chemotherapy (not specified)	NR	NR
Jeong, 2015 [75]	NR	NR	NR
Kaito, 2017 [40]	Adjuvant: S-1, XELOX, S-1+cisplatin, S-1+oxaliplatin (SOX)	NR	NR
Kalinka-Warzocha, 2015 [87]	Various chemo (27 regimens; DCF common). G-CSF prophylaxis studied	I–IV	Some G-CSF-related AEs (bone/back pain, leukocytosis), overall low incidence
Kang, 2017 [48]	NR	NR	NR
Kim, 2021 [65]	Crizotinib (MET inhibitor)	I	NR
Kim, 2020 [52]	Chemotherapy (not specified)	NR	NR
Kim, 2019 [80]	Palliative chemotherapy	I, II	NR
Kim, 2018 [36]	Adjuvant: S-1 vs XELOX	NR	NR
Kim, 2016 [77]	Chemotherapy for *H. pylori* eradication vs placebo	NR	NR
Li (J), 2018 [43]	NR	NR	3 complications in MIS group vs 8 in open surgery
Li (Q), 2018 [66]	Trastuzumab + chemotherapy (platinum-FP or taxane-FP, etc.)	I	Neutropenia, leukopenia most common
Li, 2020 [67]	Trastuzumab + platinum-FP or taxane-FP; maintenance: Trastuzumab alone vs Trastuzumab + single chemotherapy	I–III	Hematologic (neutropenia, thrombocytopenia, anemia), non-hematologic (anorexia, infection)
Liu, 2015 [81]	IIC	NR	Organ/space SSI
Martinez-Lago, 2015 [63]	Radiochemotherapy: 5-FU + leucovorin, then more 5-FU	NR	Neutropenia, anemia, thrombocytopenia, diarrhea, mucositis, hand-foot syndrome (mostly grade II–III, none grade IV)
Mokdad, 2018 [26]	Various chemotherapies ± chemoradiotherapy	NR	NR
Murat Sedef, 2019 [62]	(5-FU + cisplatin) ± taxanes	NR	NR
Narita, 2023 [71]	Cytotoxic chemotherapy: irinotecan, oxaliplatin combos, FTD/TPI, etc.	NR	Neutropenia, thrombocytopenia, anemia, GI issues, neuropathy, rash, hypothyroidism, pneumonitis, etc.
Noh, 2018 [79]	Chemotherapy (not specified)	NR	NR
Nomura, 2019 [49]	Chemotherapy (not specified)	I	Remnant group: 6 bleeds, 3 perforations; Intact group: 174 bleeds, 55 perforations
Oh, 2021 [57]	S-1 monotherapy or XELOX	NR	NR
Olmi, 2020 [42]	NR	NR	34 total complications: 17 surgical (fistulas=7), 19 medical (17 transfusions)
Oyama, 2013 [38]	S-1 + cisplatin, antiemetics (aprepitant, granisetron, dexamethasone)	I	Nausea, vomiting, anorexia
Oyama, 2016 [39]	S-1 + cisplatin; antiemetics (oral aprepitant, IV dexamethasone, palonosetron)	NR	Anorexia, diarrhea, hiccups, constipation
Petrioli, 2020 [55]	Neoadjuvant: DOC or EOF	I	Neutropenia, stomatitis, nausea/vomiting more frequent in EOF

(*continued*)

Table 4. Pharmacological interventions, treatment lines and adverse events (continued)

First Author, year	Pharmacological interventions	Treatment lines	Adverse events
Pyo, 2016 [19]	NR	NR	Early complications higher in ESD (9.0%) vs surgery (6.6%), late complications higher in surgery (2.9% vs 0.5%).
Qiu, 2023 [72]	Apatinib 250–500 mg/day	II+	Hypertension, hand-foot syndrome, proteinuria, fatigue, hematologic
Qiu, 2014 [21]	XELOX induction; maintenance with capecitabine or observation	I	Neutropenia, thrombocytopenia, anemia, leukopenia, fatigue, anorexia, nausea, mucositis, hand-foot syndrome, neuropathy
Rausei, 2015 [54]	NAC regimens: ECF, EOX, or FOLFOX	I	NR
Saito, 2021 [27]	IP paclitaxel (40 mg/m^2 d1,8) + IV oxaliplatin (100 mg/m^2 d1) + S-1 (14 on/7 off)	NR	Leukopenia, neutropenia, anemia, thrombocytopenia, fatigue, anorexia, GI AEs, neuropathy, infection
Sarriugarte, 2018 [41]	Preoperative chemotherapy for cT>1 (FLOT or similar)	NR	NR
Sato, 2020 [78]	NR	NR	NR
Shi, 2021 [28]	IP paclitaxel 40 mg/m^2 d1,8 + IV oxaliplatin 100 mg/m^2 d1 + S-1 80 mg/m^2 (14 on/7 off)	NR	Leukopenia, neutropenia, anemia, thrombocytopenia, neuropathy, diarrhea, nausea, vomiting
Shin, 2024 [76]	NR	NR	Bleeding (main ESD complication)
Tate, 2019 [20]	NR	NR	Delayed bleeding, hospital admission, severe AEs within 30 days
Terashima, 2021 [83]	Postoperative chemotherapy (not specified)	NR	NR
Trip, 2014 [61]	NR	NR	Nephrotoxicity
Ushiku, 2015 [82]	NR	NR	NR
Wang, 2017 [58]	Chemotherapy alone vs chemotherapy + CIT	NR	Few chemotherapy-related myelosuppression with CIT
Wang, 2020 [44]	NR	NR	NR
Yamamoto, 2020 [88]	Vonoprazan 20 mg pre-ESD + IV omeprazole 20 mg same evening	NR	Delayed bleeding incidence
Yan, 2019 [64]	IV chemotherapy (oxaliplatin, leucovorin, tegafur) ± sequential S-1	NR	Anemia, leukopenia, thrombocytopenia, liver dysfunction, diarrhea, GI reaction
Yang, 2015 [47]	NR	NR	Post-ESD bleeding linked to antithrombotic use; low perforation/pneumonia
Zhang, 2019 [34]	IP hyperthermic perfusion (cisplatin 50 mg/m^2 at 42°C × 60 min)	I	Fewer fevers in IPHP group; no increase in major complications
Zhang, 2024 [73]	Combination immunotherapy ± targeted therapy ± chemotherapy, or immunotherapy combos	I, II, III+	NR

AEs, adverse events; BSC best supportive care; CAPOX/ CAPEOX/ XELOX, capecitabine + oxaliplatin; CF, cisplatin + 5-fluorouracil; CIT, cellular immunotherapy; DCFm, docetaxel + cisplatin + 5-fluorouracil; DOC , docetaxel + oxaliplatin + capecitabine; DPYD SNPs, single nucleotide polymorphisms in dihydropyrimidine dehydrogenase gene; ECF, etoposide + cisplatin + 5-fluorouracilo; EMR, Endoscopic mucosal resection; EOF, epirubicin + oxaliplatin + 5-fluorouracil; EOX, epirubicin, oxaliplatin and capecitabine; ESD, submucosal dissection; FLOT, 5-fluorouracil + oxaliplatin + docetaxel + leucovorin; 5-FU, 5-fluorouracil; FOLFIRI, irinotecan, folinic acid, and fluorouracil; FOLFOX/ FOLFOX6, 5-fluorouracil + oxaliplatin + leucovorin; FP, fluoropyrimidine based-therapy; FTD/TPI, trifluridine/ tipiracil hydrochloride; G-CSF, granulocyte colony stimulating factor; GI, gastrointestinal; HIPEC, hyperthermic intraperitoneal chemotherapy; HIPEC, hyperthermic intraperitoneal chemotherapy; ICIs, immune checkpoint inhibitors; IIC, Intraoperative intraperitoneal chemotherapy; ILD, interstitial lung disease; IP, intraperitoneal; IV, intravenous; MET, mesenchymal-epithelial transition; MIS, minimally invasive surgery; NAC, neoadjuvant chemotherapy; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; PPI, Proton pump inhibitor; RFA, radiofrequency ablation; S-1, tegafur/gimeracil/oteracil; SOX, S-1 (tegafur/gimeracil/oteracil) and oxaliplatin; SSI, surgical site infection; TAE, transcatheter arterial embolization

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days was deemed safe and efficient [45], with double-endoscope ESD further enhancing the ability to resect difficult ulcer-scar lesions, albeit at the cost of increased procedural complexity [46]. In older patients, ESD was found to be equally safe, with no major differences in the rate of complications compared with younger patients [47]. ESD can be considered non-inferior to surgery in terms of 10-year OS, although it comes with more early complications [19]. It may be appropriate for larger lesions (over 10–15 mm) under either absolute or expanded criteria [20] and might even extend to certain small mucosal signet-ring carcinomas under strict conditions [48]. In a remnant stomach, however, ESD can take longer (remnant vs intact group: 110.3±63.9 vs 81.9±54.7; p<0.01) and achieve a lower curative resection rate (remnant vs intact group: 77.5, 107 lesions vs. 87.7%, 2,841 lesions; p<0.01), but complication rates remain comparable to those in an intact stomach [49].

Other local interventions address different clinical needs. Surgical margin length, e.g., was found not to influence 5-year OS or recurrence in T2/T3 disease, suggesting a degree of flexibility in margin settings [50]. For patients with acute, uncontrollable bleeding in advanced GC, transarterial embolization was an effective alternative when endoscopic or surgical approaches were not feasible [51]. Stenting offers another local solution, particularly for tumours in the esophagogastric junction (EGJ) or pylorus. Although overall prognosis and complication rates were similar, EGJ stents showed better stability in preventing reobstruction compared to pyloric stents. In fact, the reprocedure average period was longer in the EGJ obstruction group (158.3 ± 42.4 days vs pyloric obstruction 86.0 ± 29.1 days; p=0.022) [52].

Neoadjuvant, adjuvant, or advanced-line chemotherapy

Neoadjuvant (NAC) and adjuvant chemotherapy have demonstrated benefits in survival and surgical outcomes for GC. NAC has been associated with reduced surgical mortality and morbidity, compared to surgery alone [53, 54]. Similarly, NAC with the SOX regimen (S-1 (tegafur/gimeracil/oteracil) and oxaliplatin) achieved over 90% disease control rate and the SOX group had 3.9% metastatic lymph nodes, less than the control (9.9%), FOLFOX6 (6.6%), and XELOX (5.3%) groups [35]. The DOC-based regimen (docetaxel + oxaliplatin + capecitabine) improved 2-year progression-free survival (PFS: 54.1% vs 41.4%; p=0.14) and OS (80.8% vs 58.6%; p=0.05) compared to EOF (epirubicin + oxaliplatin + 5-fluorouracil), with a lower incidence of grade ≥3 neutropenia (23.5% vs 34.4%; p=0.33) [55]. Perioperative chemotherapy also showed a favorable trend for OS and DFS [56]. In the adjuvant context, chemotherapy proved beneficial for stage III disease in elderly patients but not for stage II [25], and XELOX (capecitabine + oxaliplatin) emerged as the recommended regimen in more advanced stages [36, 57]. Beyond these regimens, adding cellular immunotherapy to chemotherapy could further improve 3-year DFS (74.7% vs 60.6%; p=0.036) and OS (83.0% vs 64.9%; p=0.051), particularly in higher-stage disease [58].

Other authors focused on intraperitoneal approaches and hyperthermic intraperitoneal chemotherapy (HIPEC). HIPEC accelerated bowel recovery (42.9 vs 67.8 hours; p<0.05), earlier initiation of a liquid diet (3.03 vs 4.02 days; p<0.05), and reduced hospital stay (8.15 vs 14.08 days; p<0.05) compared to surgery alone [24]. For peritoneal metastases, combining SOX with intraperitoneal paclitaxel proved highly effective, often facilitating conversion surgery [27, 28]. Similarly, intraperitoneal hyperthermic perfusion (IPHP) improved 1-year survival rate (85.5% vs 73.8%; p= .027), exceeding that of non-IPHP treatment, and reduced the 2-year mortality risk by 1.8 times (OR=0.556; p=0.004) without increasing complications [34].

Beyond local or perioperative strategies, several studies highlight the utility of systemic therapy across multiple lines of treatment. Third-line chemotherapy significantly improved survival in metastatic GC, with median OS of 18 vs 8 months (p<0.0001), especially in patients under 70, with good performance status (ECOG 0–1), prior surgery, and combination first-line therapy [59]. Likewise, adding radiotherapy to chemotherapy significantly improved outcomes, with higher remission rates (90.6% vs 73.5%), longer median survival (10.6 vs 6.7 months), and better 6-month (83.3% vs 62%), 1-year (38.2% vs 22.8%), and 2-year (13.7% vs 7.6%) survival rates compared to chemotherapy alone (p<0.05) [60]. Intensity-modulated radiotherapy specifically minimized renal dose, potentially preventing long-term nephrotoxicity [61]. In distal intestinal GC, taxane-containing regimens prolonged PFS and OS by a few months [62], while infusional 5-fluorouracil (5-FU) [63] and sequential intravenous plus S-1 therapy [64] showed favorable tolerance. Maintenance capecitabine after XELOX significantly improved PFS (11.4 vs 7.1 months; p<0.001) and was identified as an independent prognostic factor, with low rates of severe side effects [21].

Targeted therapy and immunotherapy

Research on targeted therapies for specific gastric malignancies has shown how biomarkers can inform treatment strategies. Tumors with MET overactivation and a high stromal proportion had poorer overall outcomes but demonstrated improved responses to MET inhibitors like crizotinib [65]. Similarly, apatinib improved survival in advanced GC patients with poor performance status when combined with best supportive care compared to supportive care alone (4.3 vs 2.1 months; p=0.0004), with common side effects including fatigue (82.6%), appetite loss (73.9%), and anemia (69.6%) [22].

HER2-targeting has impacted on the management of GC, with trastuzumab offering substantial benefits when combined with chemotherapy. One study reported a median PFS of 7.7 months and OS of 16 months for patients receiving trastuzumab plus chemotherapy, though liver metastases or poor performance status negatively affected outcomes [66]. Maintenance therapy with trastuzumab plus single-agent chemotherapy reduced mortality risk by 29% and significantly improved OS in subgroups, including patients with stable disease (Hazard Ratio (HR)=0.084; p=0.004), age >65 (HR=0.4; p=0.015), no liver metastasis (HR=0.271; p=0.008), and fewer than two metastatic organs (HR=0.263; p=0.005). It was also more cost-efficient than trastuzumab alone [67]. Deep-learning models like Nomo-LDLM-2F can predict which patients will benefit the most from HER2-targeted therapies [37]. Furthermore, adding ramucirumab to paclitaxel nearly doubled OS compared to monotherapy (11.0 vs 5.7 months) but was associated with higher rates of grade ≥3 adverse events (60.8% vs 34.2%), particularly neutropenia (49.6% vs 8.9%) [29].

The field of immunotherapy has progressed rapidly, with ICIs becoming a critical treatment option. Multiple studies emphasize the role of predictive biomarkers: a pathomics-driven model effectively identified likely responders to ICIs [68], while a novel CT-based biomarker correlated with innate immune signaling and ICI responses in GC [69]. Interestingly, higher rates of immune-related adverse events were linked to reduced risk of death (HR=0.606, 95% CI: 0.444-0.827), suggesting a paradoxical relationship between toxicity and treatment response [70]. For heavily pretreated patients, post-nivolumab cytotoxic chemotherapy further extended survival [71], with a prognostic index identifying significantly worse outcomes in moderate- and poor-risk groups (HR=1.88 and 3.29, respectively), suggesting a potential synergistic antitumor effect warranting further investigation. Additionally, combining apatinib with p53 expression data resulted in a 17.4% objective response rate and a 79.3% disease control rate [72], while elevated alpha-fetoprotein levels were associated with poorer disease control (50.0% vs 87.7%; p<0.001), shorter PFS (p=0.011), and OS (p=0.036) during ICI therapy [73].

Prognosis, risk factors, quality of life, and supportive care

Numerous studies emphasized the impact of genetic, nutritional, and pathological factors on patient outcomes and postoperative risk. One study found that the DPYD (rs1801159) genotype was linked to a higher risk of grade 3-4 toxicity, improving the overall toxicity-risk modeling [30]. Malnutrition, or a 5% weight loss, was associated with longer hospital stays, although complication rates did not significantly change [31]. Tumour and host-related markers also played crucial roles in predicting survival: a high neutrophil-to-lymphocyte ratio, an elevated modified Glasgow Prognostic Score, poor nutritional status, and peritoneal metastases were all linked to poorer OS. The median OS was 27.6 months for the favorable-risk group, 13.2 months for the intermediate-risk group, and 8.2 months for the poor-risk group. The 2-year survival rates were 52% for the favorable-risk group, 16% for the intermediate-risk group, and 3% for the poor-risk group (p< 0.001) [74]. Logistical factors, such as post-discharge follow-up, were also important, with 16.6% of complications occurring after hospital release, particularly among patients with comorbidities or obesity [75]. In terms of endoscopic procedures, lymphovascular invasion (OR=7.636, 95% CI 1.730 to 22.857; p=0.004) and submucosal involvement (OR=3.735, 95% CI 1.026 to 12.177; p=0.047) were key indicators for lymph node metastasis, although ESD was considered safe [76].

Some studies explored the timing and detection of secondary or missed lesions, as well as other risk factors that may not necessarily affect long-term outcomes. In a large cohort, 61 missed GCs were identified, often associated with Billroth II anastomosis and PPI use. Although these cancers were typically detected at an earlier stage, their OS was similar to that of non-missed cases [23]. Moreover, *H. pylori* eradication after distal gastrectomy did not significantly affect recurrence or survival [77], and additional imaging beyond standard CT did not provide meaningful improvements in detecting advanced T3-T4 disease [78]. Interestingly, even the treatment of early-stage cancer appeared to influence long-term bone density, as patients who underwent surgical treatment experienced greater bone loss compared to those treated endoscopically. In the endoscopic group, BMD changes were –3.30% at the lumbar spine, –1.52% at the femoral neck, and 0.40% at the total hip. The gastrectomy group showed greater reductions: –7.17%, –0.30%, and –3.49%, respectively [79].

Quality of life (QoL) and supportive care measures are essential dimension of GC management. First-line chemotherapy can improve QoL, though direct comparisons among regimens remain inconclusive [80]. Despite its potential benefits in reducing peritoneal recurrence, intraoperative intraperitoneal chemotherapy was associated with a higher rate of organ/space surgical site infections (9.01% vs 3.88%; p=0.002). This results in longer hospital stay in patients who received intra-operative intraperitoneal chemotherapy (mean 20.91 days, 95% CI 19.76-22.06 vs 29.72 days, 95% CI 25.46-33.99; p=0.000) [81]. Various antiemetic strategies also play a role in patient well-being: the addition of aprepitant to cisplatin plus S-1 improved nausea control [38], while palonosetron-based prophylaxis reduced delayed emesis but did not eliminate it [39]. Several factors have been identified as contributing to an increased risk of infection (i.e., open surgery, male sex, splenectomy, higher body mass index, longer operative times) [82].

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Postoperative care significantly impacts outcomes; improving baseline and postoperative QoL, along with adjuvant chemotherapy, led to survival benefits, even in patients with incurable cases [83].

Future directions

A prominent theme emerging from the investigations is the call for larger prospective or randomized trials to better substantiate the efficacy and safety of various therapeutic approaches. The authors underscored the need for more robust trial designs to verify initial findings and address limitations often seen in smaller or retrospective studies [25, 37, 53, 61, 84]. These studies collectively argue that well-powered, multicenter research is crucial for generating stronger evidence and improving clinical decision-making in GC.

A significant concern for many researchers is the optimisation of preoperative strategies. They cautioned against sole reliance on imaging and recommended that, once NAC is completed, proceeding directly to surgery -rather than attempting second-line NAC- may improve outcomes [54]. The importance of enhanced imaging methods for more accurate T staging before NAC initiation was also emphasized [78]. Conversely, Dong (2016) [35] suggested that the SOX regimen is promising as NAC for Chinese patients with advanced GC, highlighting the need to tailor therapeutic interventions to specific demographics.

Several authors underlined the role of endoscopic or local therapies and the importance of close follow-up. Continuing endoscopic surveillance for longer than five years to detect metachronous GC was recommended [85], as well as osteoporosis screening post-endoscopic treatment to preserves bone health [79]. ESD was deemed feasible and safe for older patients [47] but additional research on ESD for small, mucosal signet-ring cell tumours is necessary [48]. To minimize diagnostic oversights, standard definition of missed GC, refined biopsy, ulcer-follow-up protocols, and caution with PPIs or Billroth II anatomy were also recommended [23].

Several studies focused on chemotherapy approaches. The importance of palliative chemotherapy in metastatic disease and ongoing guideline updates were emphasized [26]. It is imperative that a diligent monitoring system be established for the identification of adverse events. This will require the administration of a low-dose apatinib in conjunction with supportive care for patients demonstrating poor performance status [22]. Maintenance capecitabine after induction XELOX was identified as a promising strategy for advanced disease [21]. For those responding to first-line therapy, continued trastuzumab in combination with a single chemotherapy agent was recommended [67]. Similarly, the necessity for enhanced therapeutic interventions for HER2-positive cases that are complicated by liver metastases or poor performance status was underscored [66]. The authors also advocated XELOX-based adjuvant chemotherapy for stage II and for stage 3B/3C disease [36, 57]; a taxane-based triple regimen (e.g., DCF: cisplatin+5FU+docetaxel) for advanced GC was also recommended [62].

Subsequent studies addressed the development of novel therapeutic interventions, with a particular focus on immunotherapy and targeted agents, highlighting the potential for the utilisation of cellular immunotherapy in the treatment of GC and of ongoing trials to provide more definitive evidence regarding its impact [58]. Recent studies confirmed the real-world efficacy and safety of ramucirumab [29] and proposed a pathomics-based model to predict immunotherapy responses [68].

The authors discussed the importance of risk stratification, biomarkers, and supportive care. The development of more robust protocols for the prevention and management of surgical site infection is considered imperative in the event of widespread implementation of intraoperative chemotherapy [81]. A preoperative nutritional intervention to achieve optimal surgical outcomes is also important [31]. A novel surgical site infection risk model that requires validation in larger cohorts was proposed in a study [82]. Regarding molecular and histologic markers, there is a need for validation of a genotype-based nomogram [30].

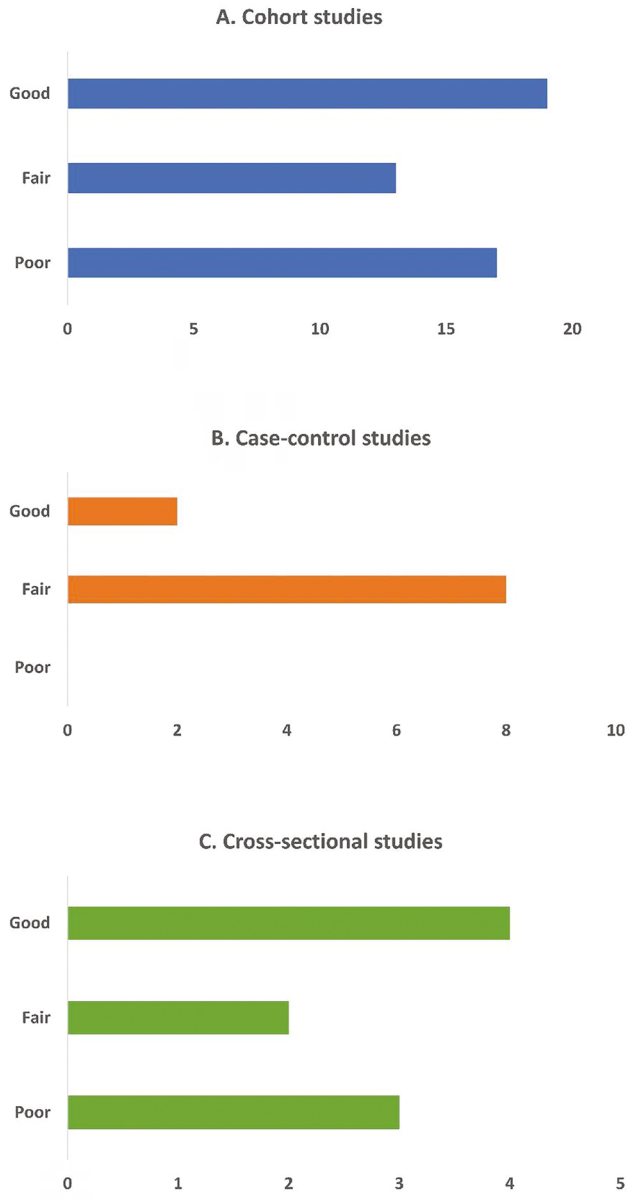
Results of the quality assessment

Of the 68 studies analyzed, 25 (36.8%) were considered to be of higher quality. Forty-nine studies employed a cohort design, with 19 of them rated as good quality, scoring between 6-8 points (Figure 2A). Most of the cohort studies with lower scores did not earn points in the comparability section and lacked representativeness of the exposed cohort. Among the 10 case-control studies (Figure 2B), two were classified as good quality, achieving 7-8 points. The remaining eight studies, rated as "fair," mainly suffered from issues with control selection and definition. The nine cross-sectional studies displayed similar quality, with two receiving low ratings due to the failure to adjust for sex or other demographic factors in the statistical analysis (Figure 2C).

DISCUSSION

The systematic review highlighted recent advances that have been achieved in GC treatment in the last decade. However, continued research and development are essential, as GC remains a significant clinical challenge. Ongoing studies are exploring future therapies, aiming to optimize combinations and sequences of existing treatments while incorporating innovative approaches. Some of the promising areas of investigation are immunotherapy (e.g., checkpoint inhibitors, cancer vaccines), targeted therapy (e.g., HER2-targeted treatments), angiogenesis inhibitors

Figure 2. Quality assessment of the gastric cancer studies



targeting blood vessel formation (e.g., ramucirumab), combination therapies that combine immunotherapy with chemotherapy or targeted therapies to enhance efficacy. In the field of personalized medicine, biomarker-driven approaches are being investigated to tailor treatments based on the genetic and molecular profile of individual tumours, improving treatment effectiveness. Research is also ongoing to evaluate the effectiveness of chemotherapy, radiotherapy, and targeted therapies when given before (neoadjuvant) or after (adjuvant) surgery. These research studies hold promise for enhancing outcomes in GC patients, with ongoing clinical trials being essential for refining and validating these potential therapies [86].

Most authors emphasized the necessity of large randomized controlled trials (RCTs) to validate current findings in GC research [32, 33, 34, 40, 41, 58, 72, etc.]. The recommendation for large RCTs is well-founded, as our review identified variability in participant numbers and study timelines. These differences reflect the diverse objectives of the research, some emphasizing immediate feasibility and early outcomes, while others focus on long-term follow-up and survival analysis. Additionally, variations in surgical techniques, medical environments, and patient conditions contribute to discrepancies in study results, making it challenging to generalize findings across different time periods and geographic regions. To address these challenges, extensive translational research, preclinical investigations, and multi-omics-based clinical trials with extended follow-up are needed to enhance consistency and applicability.

Although numerous studies have explored complex treatment regimens, including mono-immunotherapy, dual checkpoint inhibitors, and biomarker-directed therapies, the challenge of identifying the optimal treatment strategy, particularly for advanced GC, remains unresolved. The emerging therapies for GC offer several advantages and potential improvements over standard treatments but also present unique challenges and side effects. Authors emphasize the need for robust management protocols to enhance patient outcomes [29, 35, 59, 60, 68, 69, 87, 88]. Regular monitoring and supportive care are essential for mitigating side effects, while personalized treatment plans can help minimize risks, particularly for high-risk patient groups [22, 38, 39].

This systematic review has some limitations, primarily the inclusion of records published only between 2013 and 2024. While this may have excluded some relevant studies, our aim was to capture advancements in GC management over the past decade. The selection of English and French was based on the authors' language proficiency; however, as most scientific literature is published in English, no French articles were identified. Additionally, a meta-analysis was not performed due to the high heterogeneity among the studies, making a narrative synthesis a more suitable approach.

In conclusion, current findings highlight a paradigm shift toward more precise, biomarker-guided care in advanced GC, while minimally invasive or localized strategies—alone or combined with neoadjuvant and adjuvant chemotherapy—have shown promise in early GC. Optimized diagnosis and treatment may be achieved through artificial intelligence, enhanced cancer registry databases, and genome analysis to predict cytotoxic drug efficacy, ultimately improving patient prognosis. While emerging therapies offer significant potential, their effectiveness compared to existing treatments remains under investigation. Each therapeutic approach presents unique benefits and risks, underscoring the need for personalized treatment strategies that consider tumor characteristics, patient performance status, and individual preferences. As research advances, integrating these novel therapies into standard GC care could improve survival rates and QoL for patients.

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AUTHOR CONTRIBUTION

Conceptualization: IE, BU; Methodology: IE, BU, IG, YLS; Formal analysis: BU, YLS; Investigation: IE, BU, IG, YLS; Writing - Original Draft: BU, YLS, IE; Writing – Review & Editing: BU, YLS, IE, IG; Supervision: BU. All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Per- and poly-fluoroalkyl substances (PFAS) Exposure and risk of bladder and prostate cancers: A systematic review and meta-analysis

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ABSTRACT

Objectives: PFAS are synthetic chemicals that humans may be exposed to through workplace or the environment. Previous studies have suggested a carcinogenic effect. In our review, we investigated the association between PFAS exposure and risk of bladder and prostate cancer.
Methods: We searched through IARC Monographs, ATSDR documents, and PubMed (up to January 2024) to find studies that examined the relationship between PFAS exposure and bladder and prostate cancer. Four reviewers independently screened studies, extracted data, and evaluated quality using a modified version of the Newcastle-Ottawa Scale (NOS). We conducted meta-analyses using random-effects models, stratified analyses, dose-response assessments, and evaluated publication bias.
Results: We included 21 independent studies in our meta-analysis. The findings didn't reveal an association between PFOA, PFOS, and PFAS exposure and bladder cancer, as well PFOA, PFNA and prostate cancer. However, we found an association between prostate cancer and total PFAS (RR = 1.12, 95% CI =1.06–1.18), based on two studies, and an association of borderline statistical significance with PFOS (RR = 1.04, 95% CI =0.98–1.11). There was no difference between outcome, region, year of publication, study design, quality score, and gender, exposure source and different levels of PFAS for both cancer types. Publication bias was excluded for prostate cancer studies (P = 0.71) and bladder cancer (P = 0.79).
Conclusion: Our research did not find a link between different types of PFAS exposure and bladder cancer. However, it supports a potential association between PFOS exposure and prostate cancer. Bias and confounding cannot be excluded.

Keywords: bladder; malignant; occupational factors; prostate; Perfluorooctanoic Acid; PFAS; Perfluorooctane sulfonic acid.

INTRODUCTION

Per- and polyfluoroalkyl substances, or PFAS, are a large group of manufactured compounds and synthetic chemicals used in various industries since the 1940s.[1] Common types include perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorobutane sulfonate (PFBS), perfluorohexanesulfonic acid (PFHxS) [2]. They are used in products like water-resistant fabrics, paints, and cleaning products [3]. Exposure can occur through water, air, and soil. Some PFAS are classified either as carcinogenic to humans (group 1) like PFOA or as possibly carcinogenic to humans (group 2B)

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like PFOS, particularly due to their association with kidney and testicular cancers according to the International Agency for Research on Cancer (IARC) reports in 2017 and 2024 [4,5]. Limited evidence also suggests an association with other cancer types [6,7].

Urinary cancers, including bladder and other urinary tract cancers (without kidney), account for about 3% of cancers worldwide, with a higher incidence in high-income countries (age-standardized rate [ASR]: 5.6 per 100,000). Globally, urinary cancers occur more frequently in men than in women, with a ratio of 2.5:1. Additionally, prostate cancer is the third most diagnosed malignancy, with 1,414,259 cases, making up 7.3% of the total cases and significantly impacting the male population [8].

Many factors have been linked to the development of bladder cancer, including occupational, lifestyle, and genetic factors. These include smoking, being overweight, lack of physical activity, uncontrolled hypertension, alcohol consumption, diet, and Schistosoma infection. Occupational exposures also play a significant role, particularly for men in high-risk job titles such as painters, machinists, printers, firefighters, hairdressers, and truck drivers who are exposed to substances like pesticides, chromium, aromatic amines, coal tars and pitches, polyaromatic hydrocarbons, and diesel engine exhaust [9–11]. Conversely, knowledge on modifiable risk factors of prostate cancer remains limited. However, previous individual epidemiologic studies have not definitively established whether elevated levels of PFOA and other PFAS are associated with prostate and bladder cancer incidence or mortality [12].

In this report, we aim to conduct a systematic review and meta-analysis of occupational and environmental observational studies which evaluated the association between exposure to overall and individual type of PFAS and bladder, and prostate cancer incidence and mortality.

METHODS

Data Sources, Search Strategy, Selection Criteria, and Quality Assessment

Details of the overall project reported elsewhere [13,14]. Shortly, this report is a part of extended systematic review and meta-analysis (PROSPERO database's registration No. CRD62024560837) with focus on association between exposure to different types of PFAS, including PFOA, PFOS, PFDA, PFNA and total PFAS, and solid and non-solid cancers other than kidney, liver and testis, which were included in a previous review 6, according to the COSMOS-E and PRISMA-statements (supplementary table 1a,b) [15,16]. We conducted the literature search on January 23, 2024, for English language peer-reviewed publications in PubMed and Scopus with no limit according to year of publication to identify relevant studies and, we added the reference lists of the IARC Monograph on PFOA/PFOS [4] and the Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile of PFAS [17]. The search strategy utilized the following MeSH terms (("PFOA" OR "Perfluorooctanoic Acid" OR "PFOS" OR "Perfluorooctane Sulfonic Acid" OR "PFAS" OR "per and poly fluoroalkyl substances" AND ("cancer" OR "malignant" OR "carcinoma" OR "neoplasm" OR "tumor" OR "myeloid" OR "lymphoma" OR "Hematologic")) (the complete search string is reported in Supplementary Table 2).

We included cohort, case-control, cross sectional, and ecological human occupational and environmental studies. Studies involving animals or other non-human experimental systems were excluded. Also, we excluded studies for which we couldn't find the full text of the relevant articles. Four reviewers independently screened the titles, abstracts, and full text and extracted data.

The data extraction file contained demographic characteristics of the original studies such as the author's name, year of publication, country, study design type (cohort, case-control, ecological, and cross sectional), patient characteristics (gender), cancer type, PFAS types, PFAS exposure source (occupational or environmental), duration and level of exposure. We also extracted the effect size measures, such as relative risks (RRs), odds ratios (ORs), risk ratios, rate ratio, standardized mortality ratio (SMR), or standardized incidence ratio (SIR) as well as their respective 95% confidence intervals (CI). If results were reported only for subgroups, we combined them using a fixed effect meta-analysis. When RRs or CIs were not reported, we calculated them from the raw data if possible.

The eligible studies were critically appraised by four independent reviewers using a modified version of the Newcastle-Ottawa Scale (NOS) (Supplementary Table 3) [18] for case-control, ecological, and cohort studies. The scores were broken down into 2 categories: low quality if the study scored less than 8 and high quality if the study scored 8 or higher (Supplementary Table 4).

Statistical Analysis

Totally we identified of 39 independent studies related to different solid and non-solid cancer types other than liver, kidney and testicular cancer (Figure 1). We restricted this analysis to 21 studies reporting incidence and mortality RR and the respective 95% CIs related to bladder (n=14 studies) or prostate cancer (n=19 studies) (Figure 1) and examined their association with total and different types of PFAS. Heterogeneity among studies was assessed using the Q test, which evaluated variation across studies rather than within them, and the I^2 statistic, which indicates the percentage of variance in a meta-analysis attributable to study heterogeneity [19]. To account for heterogeneity in the design characteristics of the included studies, random-effects models were used for the meta-analysis [20]. We then conducted

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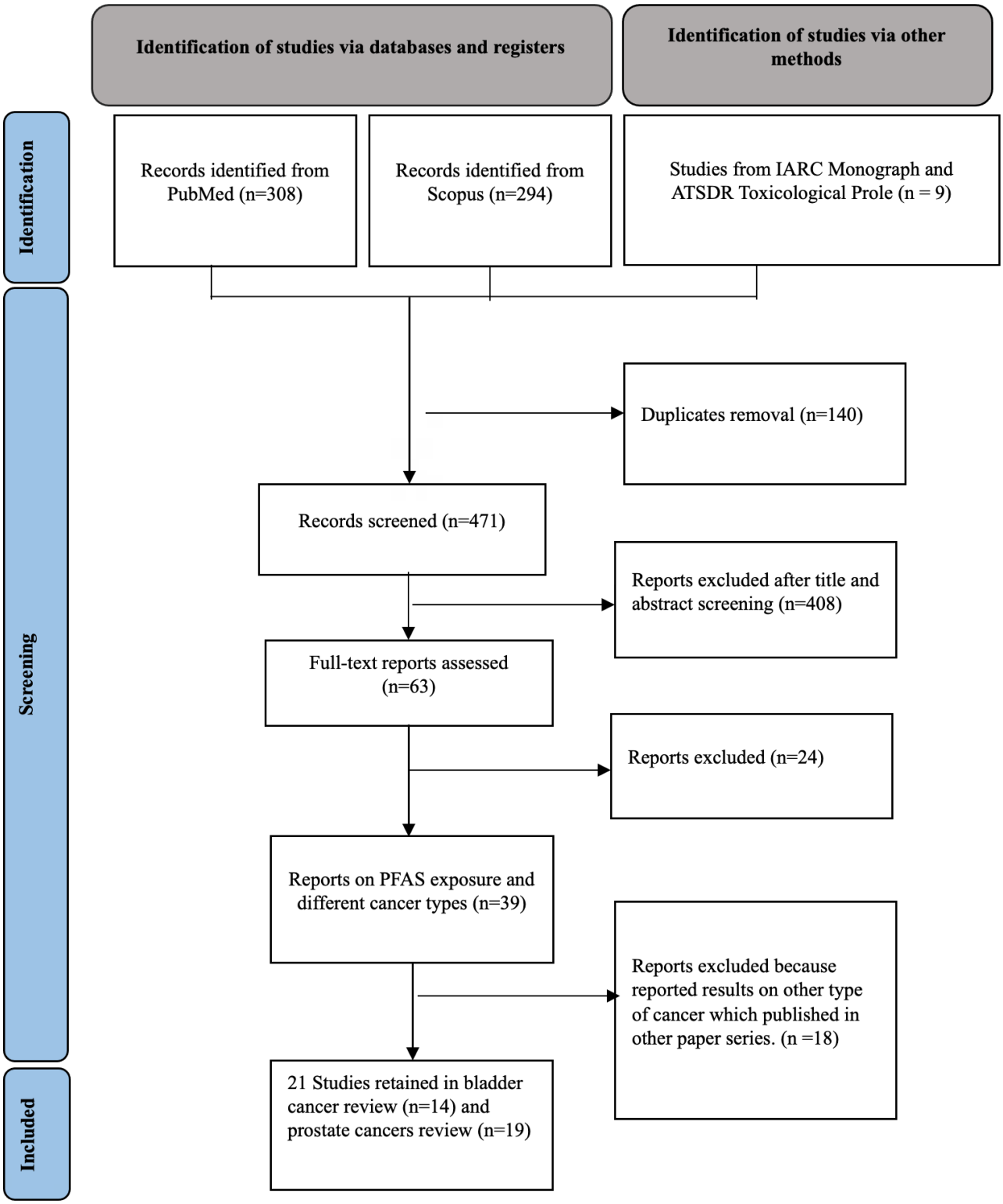
Figure 1. Selection of studies for inclusion in the review and meta-analysis



stratified analyses by region (North America, Europe, and other regions), study design (case-control, cohort, ecological), quality score (low quality or high quality), outcome (incidence or mortality), exposure source (environmental, occupational), gender (male, female, both), and year of publication (<2017 vs. ≥ 2017). We also extracted dose-response results, including analyses by level of low, medium, or high exposure (Supplementary table 5 and 6). We conducted a meta-analysis for each exposure category and performed a meta-regression of the linear trend for the respective exposure categories. Lastly, we assessed publication bias by creating a funnel plot and applying a regression asymmetry test [21]. All statistical analyses were completed using STATA version 17 (Stata, College Station, TX, USA).

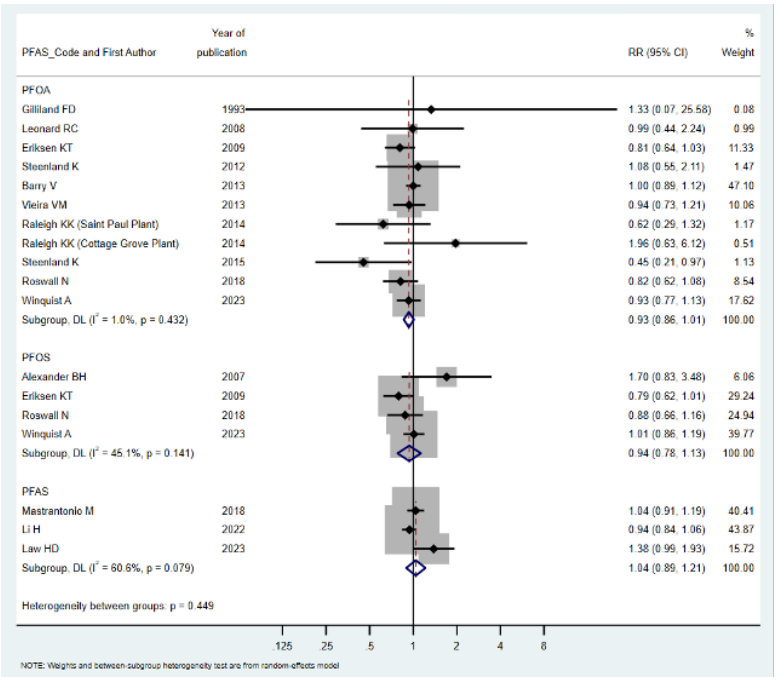
RESULTS

Among the 21 studies retained in the review, [22–42] 15 cohort, studies 5 case-control, studies and 1 ecological study reported 209 different risk estimates for bladder and prostate cancer considering different types of PFAS, genders and outcomes. Details of these studies are provided in Supplementary Table 4.

The findings revealed a lack of association between PFOA, PFOS, and PFAS exposure and bladder cancer (Figure 2a, Table 1) as well PFOA, PFNA and prostate

Figure 2. Forest plot (random-effects model) of results on the association between PFAS exposure and a) bladder cancer, b) prostate cancer

a) Bladder



b) Prostate

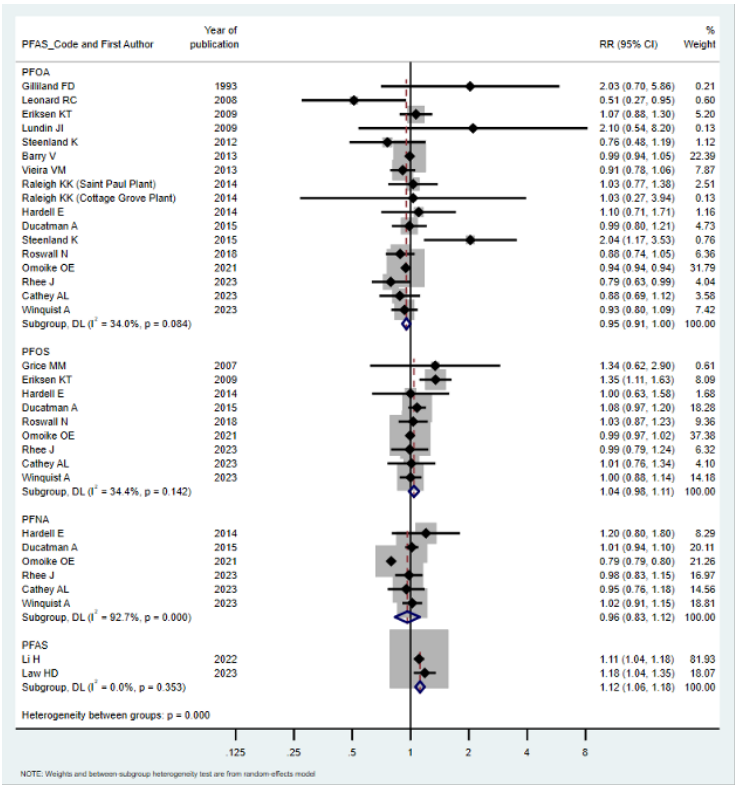


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Table 1. Results of the meta-analyses of bladder and prostate cancer stratified by region, study design, quality score, outcome, gender, year of publication

Cancer type	Characteristic	N risk estimates	RR (95% CI)	p heterogeneity
Bladder	**Overall**			
	Region			
	North America	11	0.98(0.91-1.06)	0.05
	Europe	6	0.91(0.83-1.00)	
	Other regions	1	1.38(0.99- 1.93)	
	Study design			
	Case control	1	0.94(0.73-1.21)	0.44
	Cohort	17	0.94(0.87-1.02)	
	Ecological	1	1.04(0.91-1.19)	
	Quality score			
	Low quality (< 8)	10	0.98(0.87-1.09)	0.58
	High quality (>= 8)	8	0.93(0.86-1.02)	
	Years of puplication			
	<2017	11	0.91(0.80-1.04)	0.35
	>=2017	7	0.98(0.90-1.06)	
	Outcome			
	Incidence	12	0.97(0.89- 1.05)	0.21
	Mortality	6	0.85(0.71-1.02)	
	Gender			
	Men	4	0.99(0.90-1.09)	0.27
	Women	3	0.84(0.69-1.00)	
	Both	17	0.95(0.89-1.02)	
	Exposure			
	Occupational	7	0.96(0.64-1.45)	0.96
	Environmental	11	0.96(0.89-1.02)	
	Type of PFAS			
	PFOA	11	0.93(0.86- 1.01)	0.45
	PFOS	4	0.94(0.78- 1.13)	
	PFAS	3	1.04(0.89-1.21)	
	PFOA			
	Region			
	North America	9	0.96(0.88- 1.05)	0.09
	Europe	2	0.81(0.68-0.97)	
	Other regions	0	-	
	Study design			
	Case control	1	0.94(0.73-1.21)	0.86
	Cohort	10	0.92(0.83- 1.01)	
	Quality score			
	Low quality (< 8)	5	0.87(0.73-1.03)	0.40
	High quality (>= 8)	6	0.95(0.85-1.04)	
	Years of puplication			
	<2017	9	0.93(0.82-1.05)	0.71
	>=2017	2	0.89(0.76-1.04)	
	Outcome			
	Incidence	6	0.92(0.81-1.05)	0.47
	Mortality	5	0.84(0.66-1.06)	
	Gender			
	Men	2	0.93(0.74-1.17)	0.99
	Women	1	0.91(0.63-1.31)	
	Both	10	0.92(0.84-1.01)	
	Exposure			
	Occupational	6	0.84(0.56-1.25)	0.59
	Environmental	5	0.94(0.86-1.02)	

(*continued*)

Table 1. Results of the meta-analyses of bladder and prostate cancer stratified by region, study design, quality score, outcome, gender, year of publication (continued)

	Characteristic	N risk estimates	RR (95% CI)	p heterogeneity
Prostate	**Overall**			
	Region			
	North America	25	0.96(0.90-1.03)	0.01
	Europe	8	1.08(0.99-1.18)	
	Other regions	1	1.18(1.04-1.35)	
	Study design			
	Case control	17	1.05(0.98-1.12)	0.09
	Cohort	21	1.04(0.99-1.10)	
	Quality score			
	Low quality (< 8)	19	1.01(0.96-1.06)	0.75
	High quality (>= 8)	11	1.02(0.95-1.10)	
	Years of puplication			
	<2017	18	1.04(0.97-1.12)	0.13
	>=2017	16	0.97(0.90- 1.04)	
	Outcome			
	Incidence	26	1.00(0.95-1.06)	0.52
	Mortality	8	0.95(0.81-1.12)	
	Exposure			
	Occupational	8	1.11(0.78- 1.56)	0.61
	Environmental	22	1.01(0.97- 1.05)	
	Type of PFAS			
	PFOA	17	0.95(0.91-1.00)	<0.001
	PFOS	9	1.04(0.98-1.11)	
	PFNA	6	0.96(0.83-1.12)	
	PFAS	2	1.12(1.06-1.18)	
	PFOA			
	Region			
	North America	6	1.00(0.97-1.02)	0.2
	Europe	3	1.14(0.93- 1.41)	
	Other regions	0	-	
	Study design			
	Case control	5	0.94(0.94- 0.94)	0.73
	Cohort	12	0.98(0.88- 1.09)	
	Quality score			
	Low quality (< 8)	9	0.92(0.84-1.01)	0.19
	High quality (>= 8)	8	0.99(0.94-1.04)	
	Years of puplication			
	<2017	12	1.00(0.90-1.10)	0.28
	>=2017	5	0.94(0.94-0.94)	
	Outcome			
	Incidence	11	0.96(0.91- 1.00)	0.59
	Mortality	6	0.90(0.71- 1.13)	
	Exposure			
	Occupational	7	1.08(0.74-1.60)	0.48
	Environmental	10	0.94(0.94-0.94)	

cancer (Figure 2b, Table 1). Conversely, we found an association between prostate cancer and total PFAS (RR = 1.12, 95% CI =1.06–1.18, 2 risk estimates), and an association of borderline statistical significance with PFOS (RR = 1.04, 95% CI =0.98–1.11, 9 risk estimates) (Figure 2b, Table 1).

Publication bias was excluded through the Egger test for prostate cancer studies (P = 0.71) and bladder cancer (P = 0.80); funnel plots are shown in Figure 3.

Results of stratified analyses according to selected characteristics are reported in Table 1. There was no difference according to outcome, region, year of publication, study design, quality score, and gender, exposure source for both cancer types. A similar stratified analysis limited to PFOA exposure showed no effect modification.

Results on different levels of PFAS exposure didn't reveal any dose trend for bladder and prostate cancer (Table 2). The relative risks of different levels of PFAS exposure from each study included in our analysis are reported in Supplementary Table 5 (for bladder cancer) and Supplementary Table 6 (for prostate cancer).

Figure 3. Funnel plot of results on the association between PFAS exposure and a) bladder cancer, b) prostate cancer

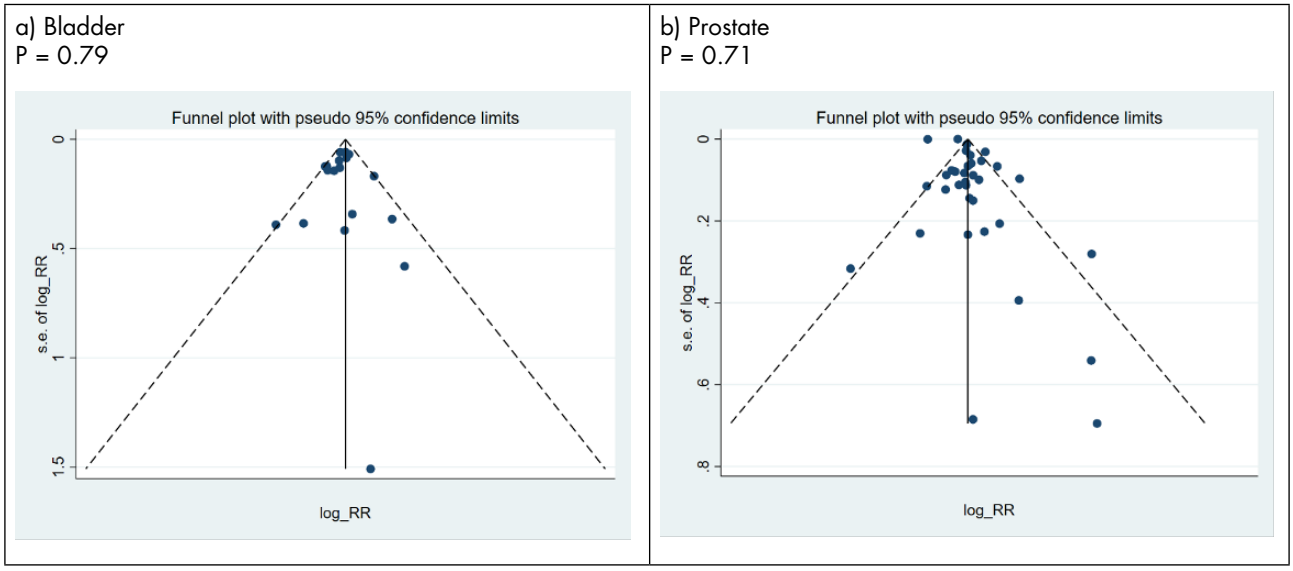

Table 2. Meta-analysis of results on level of PFAS exposure

Characteristic	PFAS type	Dose category	RR (95% CI)	p trend
Prostate	**PFOA**	Low (9 studies)	0.88(0.73-1.06)	0.85
		Medium (9 studies)	0.91(0.79-1.04)	
		High (9 studies)	0.85(0.70-1.03)	
	PFOS	Low (5 studies)	1.11(0.94-1.29)	0.82
		Medium (4 studies)	1.10(0.93-1.30)	
		High (5 studies)	1.08(0.91-1.27)	
	PFNA	Low (2 studies)	0.83(0.65-1.06)	0.27
		Medium (2 studies)	0.99(0.76-1.28)	
		High (2 studies)	1.02(0.78-1.33)	
Bladder	**PFOA**	Low (7 studies)	0.83(0.67-1.03)	0.92
		Medium (7 studies)	0.94(0.77-1.15)	
		High (7 studies)	0.84(0.63-1.10)	
	PFOS	Low (4 studies)	1.01(0.70-1.47)	0.36
		Medium (3 studies)	0.98(0.77-1.26)	
		High (4 studies)	0.82(0.58-1.16)	
* The p-value of test for linear trend.				

DISCUSSION

Our systematic review and meta-analysis found no association between PFAS exposure and risk of bladder cancer, but we found a borderline association in studies which focused particularly on prostate cancer and PFOS exposure. In addition, there was an association between total PFAS exposure and prostate cancer, which however was based on two studies only.

Previous individual reports, included in our review, have shown that there is a correlation between exposure to certain types of PFAS and the development of prostate [27,32,43] and bladder [44] cancers in specific populations. While the precise way in which PFAS contributes to cancer is not completely understood, some studies have suggested various possible mechanisms. Initially, their potential impact on the normal human prostate stem-progenitor cells (SPCs) may lead to altered transcriptomes and metabolomes, potentially promoting a preneoplastic state and increasing the risk of prostate cancer with prolonged exposure [45]. Additionally, a hormone-dependent effect of PFAS has been suggested. Apart from disrupting hormones and damaging DNA, PFAS can also cause inflammation and raise levels of related markers in the body, which may ultimately contribute to the development of cancer [54]. These findings offer valuable insights into the potential mechanisms by which PFAS could influence the development of prostate and bladder cancer [46,47]. However, if present, the carcinogenic effect is not supported by strong mechanistic evidence and needs more future studies.

Since certain PFAS are primarily eliminated from the body through urine, this can result in prolonged exposure of the kidneys and other urinary organs to these chemicals. Also, previous research has specifically documented their possible impact on the incidence of kidney cancer, which is attributed to oxidative stress and epigenetic mechanisms associated with tubular reabsorption [48,49]. However, we did not encounter the same evidence of a possible association with bladder cancer. This might be because the concentration of PFAS in the urine collected in the bladder is lower, and the urine is expelled from the body after a short period of time [50]. Another reason for this is that most cohort studies, which primarily focused on mortality, did not have direct measurements of PFAS. These studies typically only examined a few major PFAS and had a limited number of specific cancer cases. Additionally, many of these studies lacked proper control groups and did not consider the potential influence of positive or negative confounding factors. There is a lack of published evidence from ongoing large-cohort studies.

Our systematic review and meta-analysis had certain limitations. The primary concern was the scarcity of studies available, especially those focusing on the impact of exposure to specific PFAS compounds other than PFOA or PFOS, the limited information on dose-response, and the lack of details on clinical aspects such as muscle invasiveness for bladder and tumor grade for prostate cancer, and the low power of stratified analyses. Additionally, it is crucial to consider various confounding factors that can impact the outcomes of studies on bladder and prostate cancer. These factors should be carefully considered when interpreting the results. For bladder cancer, factors such as tobacco smoking, being overweight, having diabetes, and a lack of physical activity should be taken into consideration [51]. When it comes to prostate cancer, age, race, family history, and genetics (such as the BRCA1 or BRCA2 gene variant) can also play a role [52]. However, it is worth noting that many of the studies included in our review, especially cohort studies, did not adjust for potential non-occupational and occupational confounders. This is especially important for bladder cancer, as 17 out of 19 relative risks were extracted from cohort studies. It is important to acknowledge that case-control studies may introduce other types of bias [53]. The stratification analysis we conducted did not reveal any heterogeneity in the results based on the characteristics we considered. The lack of studies from regions like Latin America, East Asia, and sub-Saharan Africa was another limitation. As a last note, along with reporting the dose of exposure, working on duration particular to evaluate long-term and chronic effects of PFAS can be helpful, which are not addressed in most studies.

In spite of these limitations, to the best of our knowledge, this systematic review and meta-analysis represents the first comprehensive examination of the potential link between environmental and occupational exposure to PFAS and bladder and prostate cancer. Thus, it may be useful to summarize the limitations of different reports and to suggest improvements for future research.

In conclusion, our research did not find a link between different types of PFAS exposure and bladder cancer. However, it supports a possible association between PFOS exposure and prostate cancer, not supported by limited dose-response results. Bias and potential confounding cannot be excluded.

ABBREVIATIONS

Agency for Toxic Substances and Disease Registry, ATSDR; Endocrine-disrupting chemicals, EDCs; International Agency for Research on Cancer, IARC; Nitrogen dioxide, NO_2; Odds ratio, OR; Risk ratio, rate ratio, RR; Standardized mortality ratio, SMR; Standardized incidence ratio, SIR; Stem-progenitor cells, SPCs; Perfluorooctanoic Acid PFOA; Per- and polyfluoroalkyl substances, PFAS; Perfluorooctane sulfonic acid, PFOS; Perfluorononanoic acid, PFNA; Perfluorobutane sulfonate, PFBS; Perohexanesulfonic acid, PFHxSrfluo

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The study was conducted with internal resources of the participating institutions.

DATA AVAILABILITY STATEMENT

The data supporting this study's findings are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

PB and TZ and MSS conceived and designed the study, MSS, EK, and SZ selected the studies and extracted the data, MSS conducted the statistical analysis; MSS and EK drafted the manuscript; SZ, TZ, and PB provided substantial comments to the results and manuscript.

CONFLICTS OF INTEREST

PB acted as an expert in litigation involving PFAS exposure, unrelated to the present work. Other authors declare no conflict of interest.

ETHICS APPROVAL STATEMENT

No Ethics Approval

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LIST OF SUPPLEMENTARY TABLES

Number	Title
Supplementary Table 1a	PRISMA Checklist
Supplementary Table 1b	PRISMA Abstract Checklist
Supplementary Table 2	Detailed search strategy used on the different databases.
Supplementary Table 3	NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
Supplementary Table 4	Selected characteristics of the studies included in the review and meta-analysis.
Supplementary Table 5	Relative risk of bladder cancer by level of PFAS exposure
Supplementary Table 6	Relative risk of prostate cancer by level of PFAS exposure

APPENDIX

Supplementary Table 1a. PRISMA Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			p1, line2
Title	1	Identify the report as a systematic review.	P1, line 3
ABSTRACT			P2, line 43
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	-
INTRODUCTION			P3, line 86
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	P3, line 88–112
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	P3, line 113–115
METHODS			P4, line 122
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	P4, lines 139–143
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	p 4, lines 129–133
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	p, line 134–138, and supplementary table2
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	p4 and figure 1
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	P4, line 144–152
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	P4, line 144–152
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	P4, line 144–152
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	P 4, lines 153–156
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	N/A

(*continued*)

Supplementary Table 1a. PRISMA Checklist (continued)

Section and Topic	Item #	Checklist item	Location where item is reported
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	figure 1
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	p 4, lines 150–152
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	N/A
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	P5, lines 161–177
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	P5, lines 166–175
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	N/A
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	P 5, lines 175
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	N/A
RESULTS			P 6
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	p6, lines 193–197
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	p6, lines 193. and supplementary table 4
Study characteristics	17	Cite each included study and present its characteristics.	supplementary table 4
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	p6, lines 201, and figure 3
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimates and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	N/A
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	supplementary table 4
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	P6, lines 198–202Figure 2
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	p6, lines 203–209, and table 1
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	N/A
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	p6, lines 200–202, and figure 3
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	N/A

(*continued*)

Supplementary Table 1a. PRISMA Checklist (continued)

Section and Topic	Item #	Checklist item	Location where item is reported
DISCUSSION			P7
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	P7, lines 210–236
	23b	Discuss any limitations of the evidence included in the review.	P7–8, lines 249–236
	23c	Discuss any limitations of the review processes used.	P7–8, lines 249–236
	23d	Discuss implications of the results for practice, policy, and future research.	p8, lines 168–272
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	p4, line 124–127
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	N/A
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	p4, line 124–127
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	p1, line 22
Competing interests	26	Declare any competing interests of review authors.	P1, line30
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	P1, line 32

Supplementary Table 1b. PRISMA Abstract Checklist

Section and Topic	Item #	Checklist item	Reported (Yes/No)
TITLE			
Title	1	Identify the report as a systematic review.	Yes
BACKGROUND			
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	Yes
METHODS			
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	Yes
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	Yes
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	Yes
Synthesis of results	6	Specify the methods used to present and synthesise results.	Yes
RESULTS			
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	Yes
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	Yes

(*continued*)

Supplementary Table 1b. PRISMA Abstract Checklist (continued)

Section and Topic	Item #	Checklist item	Reported (Yes/No)
DISCUSSION			
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	Yes
Interpretation	10	Provide a general interpretation of the results and important implications.	Yes
OTHER			
Funding	11	Specify the primary source of funding for the review.	N/A
Registration	12	Provide the register name and registration number.	No

Supplementary Table 2. Detailed search strategy used on the different databases.

Database	Search string
PubMed	(("PFOA"[Text Word] OR "Perfluorooctanoic Acid"[Text Word] OR "PFOS"[Text Word] OR "Perfluorooctane Sulfonic Acid"[Text Word] OR "PFAS"[Text Word] OR "per and poly fluoroalkyl substances"[Text Word]) AND ("cancer"[Text Word] OR "malignant"[Text Word] OR "carcinoma"[Text Word] OR "neoplasm"[Text Word] OR "tumor"[Text Word] OR "myeloid"[Text Word] OR "lymphoma"[Text Word] OR "Hematologic"[Text Word])) AND (humans[Filter])
Scopus	(TITLE-ABS-KEY ("PFOA") OR TITLE-ABS-KEY ("Perfluorooctanoic Acid") OR TITLE-ABS-KEY ("pfosa") OR TITLE-ABS-KEY ("Perfluorooctane Sulfonic Acid") OR TITLE-ABS-KEY ("pufas") OR TITLE-ABS-KEY ("per and poly fluoroacyl substances")) AND (TITLE-ABS-KEY("cancer") OR TITLE-ABS-KEY("malignant") OR TITLE-ABS-KEY("carcinoma") OR TITLE-ABS-KEY("neoplasm") OR TITLE-ABS-KEY("tumor") OR TITLE-ABS-KEY("myeloid") OR TITLE-ABS-KEY("lymphoma") OR TITLE-ABS-KEY("Hematologic")) AND (LIMIT-TO (SRCTYPE,"j")) AND (LIMIT-TO (DOCTYPE,"ar")) AND (LIMIT-TO (LANGUAGE,"English")) AND (LIMIT-TO (EXACTKEYWORD,"Human") OR LIMIT-TO (EXACTKEYWORD,"Humans") OR LIMIT-TO (EXACTKEYWORD,"Male") OR LIMIT-TO (EXACTKEYWORD,"Female")) AND (EXCLUDE (SUBJAREA,"ARTS") OR EXCLUDE (SUBJAREA,"EART") OR EXCLUDE (SUBJAREA,"SOCI") OR EXCLUDE (SUBJAREA,"VETE") OR EXCLUDE (SUBJAREA,"MATE") OR EXCLUDE (SUBJAREA,"ENGI") OR EXCLUDE (SUBJAREA,"COMP") OR EXCLUDE (SUBJAREA,"CENG") OR EXCLUDE (SUBJAREA,"MULT") OR EXCLUDE (SUBJAREA,"BIOC") OR EXCLUDE (SUBJAREA,"PHAR") OR EXCLUDE (SUBJAREA,"NURS") OR EXCLUDE (SUBJAREA,"AGRI") OR EXCLUDE (SUBJAREA,"IMMU") OR EXCLUDE (SUBJAREA,"CHEM") OR EXCLUDE (SUBJAREA,"NEUR") OR EXCLUDE (SUBJAREA,"PSYC") OR EXCLUDE (SUBJAREA,"DENT") OR EXCLUDE (SUBJAREA,"PHYS"))

Supplementary Table 3

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
CASE CONTROL STUDIES (maximum score: 9)
Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?
a) yes, with independent validation **(1)**
b) yes, eg record linkage **(1)** or based on self-reports **(0.5)**
c) no description **(0)**

2) Representativeness of the cases
a) consecutive or obviously representative series of cases **(1)**
b) potential for selection biases or not stated **(0)**

3) Selection of Controls
a) community controls **(1)**
b) hospital controls **(0.5)**
c) no description **(0)**

4) Definition of Controls
a) no history of disease (endpoint) **(1)**
b) no description of source **(0)**

Comparability

1) Comparability of cases and controls on the basis of the design or analysis
a) study controls for age, gender, province **(0)**
b) study controls for age, gender, province +smoking **(1)**
c) study controls for age, gender, province +smoking + other additional factors **(2)**

Exposure

1) Ascertainment of exposure
a) secure record (eg surgical records) **(1)**
b) structured interview where blind to case/control status **(1)**
c) interview not blinded to case/control status **(0.5)**
d) written self-report or medical record only **(0.5)**
e) no description **(0)**

2) Same method of ascertainment for cases and controls
a) yes **(1)**
b) no **(0)**

3) Non-Response rate
a) one or both groups over 90% **(1)**
b) one or both groups between 60- 90% **(0.5)**
c) one or both groups under 60% **(0)**
d) no statemen **(0)**

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
COHORT STUDIES (maximum score: 10)

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability.

Selection

1) Representativeness of the exposed cohort
a) truly representative of the average _____ (describe) in the community **(2)**
b) somewhat representative of the average _____ in the community **(1)**
c) selected group of users eg nurses, volunteers **(0.5)**
d) no description of the derivation of the cohort **(0)**

(*continued*)

Supplementary Table 3 (continued)

2) Selection of the non-exposed cohort
a) drawn from the same community as the exposed cohort **(1)**
b) drawn from a different source **(0.5)**
c) no description of the derivation of the non-exposed cohort **(0)**

3) Ascertainment of exposure
a) secure record (eg surgical records) **(1)**
b) structured interview **(1)**
c) written self-report **(0.5)**
d) no description **(0)**

4) Demonstration that outcome of interest was not present at start of study
a) yes **(1)**
b) no **(0)**

Comparability
1) Comparability of cohorts on the basis of the design or analysis
a) study controls for age, gender, province **(0)**
b) study controls for age, gender, province +smoking **(1)**
c) study controls for age, gender, province +smoking + other additional factors **(2)**

Outcome
1) Assessment of outcome
a) independent blind assessment **(1)**
b) record linkage **(1)**
c) self-report **(0.5)**
d) no description **(0)**

2) Was follow-up long enough for outcomes to occur
a) yes (select an adequate follow up period for outcome of interest) **(1) (average 15 years)**
b) no **(0)**

3) Adequacy of follow up of cohorts
a) complete follow up - all subjects accounted for over 90% **(1)**
b) subjects lost to follow up unlikely to introduce bias - small number lost - > ____ % (select an adequate %) follow up, or description provided of those lost) between 60- 90% **(0.5)**
c) follow up rate < ____% (select an adequate %) and no description of those lost under 60% **(0)**
d) no statemen **(0)**

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Supplementary Table 4. Selected characteristics of the studies included in the review and meta-analysis

Ref.	First author, Year of publication	Country	Study type	Measurement source	Gender	Exposure source	PFAS type	Cancer type	Outcome	Adjusted list other than gender and age, calendar period	Quality score
22	Gilliland FD, 1993	US - Minnesota	Cohort	Job History	Male	Occupational	PFOA	Prostate, Bladder	Mortality	Race	8
23	Grice MM, 2007	US - Alabama	Cohort	Questionnaire	Male	Occupational	PFOS	Prostate	Mortality	–	4.5
24	Alexander BH, 2007	US - Minnesota	Cohort	Work History	Both	Occupational	PFOS	Bladder	Incidence	–	5.5
25	Leonard RC, 2008	US - West Virginia	Cohort	Serum Sample	Male, Both	Occupational	PFOA	Prostate, Bladder	Mortality	–	6.5
26	Eriksen KT,2009	Denmark	Cohort	Questionnaire	Male	Environmental	PFOA, PFOS	Prostate, Bladder	Incidence	Education, BMI, dietary fat intake, fruit and vegetable intake	9
27	Lundin JI,2009	US - Minnesota	Cohort	Work History	Male	Occupational	PFOA	Prostate	Mortality	–	8
28	Steenland K,2012	US - West Virginia	Cohort	Serum Sample	Male, Both	Occupational	PFOA	Prostate, Bladder	Mortality	–	7
29	Barry V,2013	US - West Virginia	Cohort	Serum Sample	Male, Both	Environmental	PFOA	Prostate, Bladder	Incidence	Smoking, alcohol consumption, education	8.5
30	Vieira VM,2013	US - Ohio & West Virginia	Case-Control	Serum Sample	Male, Both	Environmental	PFOA	Prostate, Bladder	Incidence	Smoking status, insurance provider	7
31	Raleigh KK ,2014	US - Minnesota	Cohort	Work History	Male	Occupational	PFOA	Prostate, Bladder	Mortality, Incidence	–	8
32	Hardell E,2014	Sweden	Case-Control	Serum Sample	Male	Environmental	PFOA, PFOS, PFAS, PFNA	Prostate	Incidence	BMI and year of blood sampling.	6.5
33	Ducatman A,2015	US - Ohio & West Virginia	cohort	Serum Sample	Male	Environmental	PFOA, PFOS, PFNA	Prostate	Incidence	–	6.5
34	Steenland K,2015	US	Cohort	Serum Sample	Male, Both	Occupational	PFOA	Prostate, Bladder	Incidence	Race, education, BMI, and time-varying smoking, alcohol consumption	7.5
35	Mastrantonio M,2018	Italy	Ecological	Drinking Water	Both	Environmental	PFAS	Bladder	Incidence	-	6.5

(*continued*)

Supplementary Table 4. Selected characteristics of the studies included in the review and meta-analysis (continued)

Ref.	First author, Year of publication	Country	Study type	Measurement source	Gender	Exposure source	PFAS type	Cancer type	Outcome	Adjusted list other than gender and age, calendar period	Quality score
36	Roswall N,2018	Denmark	Cohort	Serum Sample	Male, Both	Environmental	PFOA, PFOS	Prostate, Bladder	Mortality	Smoking status, alcohol consumption, abstainer, waist circumference, leisure-time sports, education, area-level socioeconomic status	6.5
37	Omoike OE,2021	USA	Case Control	Serum Sample	Male, Both, Female	Environmental	PFOA, PFOS, PFNA	Prostate	Incidence	Education, race/ethnicity, PIR, BMI, serum cotinine.	6.5
38	Li H,2022	Sweden	Cohort	Drinking Water	Male	Environmental	PFAS	Prostate, Bladder	Incidence	–	7.5
39	Rhee J,2023	USA	Nested Case Control	Serum Sample	Male	Environmental	PFOA, PFOS, PFNA	Prostate	Incidence	BMI, smoking status, family history of prostate cancer, history of diabetes	7
40	Cathey AL,2023	USA	Case Control	Serum Sample	Male	Environmental	PFOA, PFOS, PFNA	Prostate	Incidence	poverty-income ratio, race, education, BMI	9
41	Law HD,2023	Australia	Cohort		Male, Both	Environmental	PFAS	Prostate, Bladder	Incidence	–	6.5
42	Winquist A,2023	USA	Cohort	Serum Sample	Male, Female, Both	Environmental	PFOA, PFOS, PFNA	Prostate, Bladder	Incidence	age at serum collection, race, education, smoking status, alcohol consumption	9

BMI; Body mass index

Supplementary Table 5. Relative risk of bladder cancer by level of PFAS exposure

PFAS type	First Author, year	Exposure level	Dose detail	RR (95% CI)
PFOA	Eriksen KT, 2009	low	Q2	0.71(0.46,1.07)
		medium	Q3	0.92(0.61,1.39)
		high	Q4	0.81(0.53,1.24)
	Steenland K, 2012	low	Q1	1.24(0.15,4.47)
		medium	Q2	2.49(0.97,5.78)
		high	Q3	0.39(0.01,2.17)
		very high	Q4	0.36(0.1,2.01)
	Vieira VM, 2013	Low		0.9(0.6,1.4)
		Medium		0.9(0.6,1.4)
		High		1.2(0.8,2)
		Very high		0.62(0.2,1.5)
	Raleigh KK, 2014	Low	Cottage Grove, Q1	0.4(0.01,2.25)
		Medium	Cottage Grove, Q2	0.93(0.11,3.38)
		High	Cottage Grove, Q3	1.61(0.44,4.13)
		Very high	Cottage Grove, Q4	0.53(0.01,2.97)
	Steenland K, 2015	low	Q2	0.32(0.08,1.33)
		medium	Q3	0.95(0.28,3.14)
		high	Q4	0.23(0.05,0.93)
	Roswall N, 2018	low	Q2	1.02(0.63,1.65)
		medium	Q3	0.87(0.55,1.4)
		high	Q4	0.61(0.37,0.99)
	Winquist A, 2023	low	3.850-<5.100	0.84(0.56,1.26)
		medium	5.100-<6.300	0.87(0.58,1.3)
		high	>=6.300	0.86(0.58,1.27)
PFOS	Alexander BH, 2007	low		2.26(0.91,4.67)
		high		1.74(0.64,3.79)
	Eriksen KT, 2009	low	Q2	0.76(0.5,1.16)
		medium	Q3	0.93(0.61,1.41)
		high	Q4	0.7(0.46,1.07)
	Roswall N, 2018	low	Q2	1.17(0.72,1.87)
		medium	Q3	0.93(0.57,1.52)
		high	Q4	0.59(0.36,0.98)
	Winquist A, 2023	low	13.000-<18.000	0.81(0.54,1.21)
		medium	18.000-<25.000	1.07(0.72,1.6)
		high	>=25.000	0.96(0.64,1.44)

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Supplementary Table 6. Relative risk of prostate cancer by level of PFAS exposure

PFAS type	First Author, year	Exposure level	Dose detail	RR (95% CI)
PFOA	Eriksen KT, 2009	low	Q2	1.09(0.78,1.53)
		medium	Q3	0.94(0.67,1.32)
		high	Q4	1.18(0.84,1.65)
	Steenland K, 2012	low	Q1	1.07(0.39,2.34)
		medium	Q2	0.82(0.3,1.78)
		high	Q3	0.65(0.21,1.51)
		very high	Q4	0.57(0.16,1.46)
	Vieira VM, 2013	Low	Q1	1.1(0.8,1.5)
		Medium	Q2	0.8(0.6,1)
		High	Q3	0.8(0.5,1.1)
		Very high	Q4	1.5(0.9,2.5)
	Raleigh KK, 2014	Low	Q1	0.66(0.21,1.54)
		Medium	Q2	1.15(0.5,2.27)
		High	Q3	0.37(0.08,1.07)
		Very high	Q4	1.29(0.56,2.54)
		Low	Q1	0.34(0.25,1.6)
		Medium	Q2	1.12(0.53,2.37)
		High	Q3	0.36(0.11,1.17)
		Very high	Q4	1.32(0.61,2.84)
	Steenland K, 2015	Low	Q2	1.81(0.69,4.78)
		Medium	Q3	2.45(0.96,6.25)
		High	Q4	1.88(0.72,4.88)
	Roswall N, 2018	Low	PFOA, Q2	0.77(0.57,1.04)
		Medium	PFOA, Q3	1.02(0.76,1.38)
		High	PFOA, Q4	0.88(0.65,1.18)
	Rhee J, 2023	Low	≥2.90, <3.80	0.75(0.53,1.07)
		Medium	≥3.80, <4.67	0.72(0.49,1.07)
		High	≥4.67, <6.50	0.67(0.44,1.03)
		very high	≥6.50	0.54(0.32,0.91)
	Winquist A, 2023	Low	3.850-<5.100	0.82(0.6,1.11)
		Medium	5.100-<6.300	0.93(0.68,1.27)
		High	>=6.300	0.83(0.61,1.14)

(*continued*)

Supplementary Table 6. Relative risk of prostate cancer by level of PFAS exposure (continued)

PFAS type	First Author, year	Exposure level	Dose detail	RR (95% CI)
PFOS	Winquist A, 2023	Low	13.000-<18.000	0.94(0.7,1.26)
		Medium	18.000-<25.000	1.11(0.81,1.5)
		High	>=25.000	1.08(0.8,1.46)
	Grice MM, 2007	low	0.39-0.89 ppm	1.36(0.61,3.02)
		high	1.30-1.97 ppm	1.08(0.44,2.69)
	Eriksen KT, 2009	Low	Q2	1.35(0.97,1.87)
		Medium	Q3	1.31(0.94,1.82)
		High	Q4	1.38(0.99,1.93)
	Roswall N, 2018	Low	PFOS, Q2	1.2(0.89,1.62)
		Medium	PFOS, Q3	0.97(0.72,1.31)
		High	PFOS, Q4	0.94(0.69,1.27)
	Rhee J, 2023	Low	≥19.10, <25.50	0.93(0.64,1.37)
		Medium	≥25.50, <33.50	1.07(0.69,1.66)
		High	≥33.50, <47.12	0.88(0.53,1.46)
		very high	≥47.12	0.84(0.45,1.58)
PFNA	Rhee J, 2023	Low	≥0.3, <0.5	0.88(0.57,1.34)
		Medium	≥0.5, <0.7	0.88(0.55,1.4)
		High	≥0.7, <1.0	0.98(0.58,1.67)
		very high	≥1.0	1.05(0.58,1.91)
	Winquist A, 2023	Low	0.450-<0.630	0.81(0.6,1.09)
		Medium	0.630-<1.000	1.04(0.76,1.41)
		High	>=1.000	1.03(0.76,1.41)

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Drugs Misuse in custodial settings: a systematic review and meta-analysis

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SUMMARY

Background: Increasing evidence suggests that custodial settings are at risk for the misuse of psychoactive drugs (DM) outside their prescription. The purpose of this systematic review and meta-analysis was to determine the extent of this phenomenon of psychoactive drug misuse in prisons, exploring the classes of drugs commonly used, the characteristics of inmates who are affected, and focusing on studies that have identified the phenomenon in an evidence-based manner.
Methods: From January 1 2025 to June 1 2025, we reviewed the scientific literature following PRISMA guidelines. 8826 studies were analyzed, 6 met the inclusion criteria.
Results: The overall rate of DM was 24%. Prisoner characteristics associated with DM included mental disorders, psychoactive substance use, and individual characteristics of prisoners. Social factors associated with DM included significant problems with the law, drug dealing, and arrests.
Conclusions: The DM issue is a non-negligible public health problem in custodial settings. Related factors can be identified and preventive actions taken.

Keywords: Drug Misuse; Problematic drug use; Custodial setting; Prison; Inmates.

INTRODUCTION

In 2022, the United Nations Office on Drugs and Crime (UNODC) defined "Misuse" as the misuse, or nonmedical use of prescription drugs, i.e., we refer to the intentional repurposing of prescribed drugs outside the intended indication, or the use of prescription drugs of illicit origin [1–3]. The drugs with the greatest potential for misuse are opioids, benzodiazepines, Z-drugs, and gabapentinoids [1),(4),(5]. The UNODC 2022 Report identified the misuse of these drugs, as a growing public health threat [6]. In addition, the report listed benzodiazepines and Z drugs, used to treat insomnia and anxiety [7], as the most commonly abused prescription drugs [8]. However, given the low toxicity profile [9,10], Gabapentinoids (the analogs of γ-aminobutyric acid, GABA) (pregabalin and gabapentin) are licensed for the treatment of epilepsy, neuropathic pain, and anxiety disorder. Evidence from recent systematic reviews shows that gabapentinoids are, in some individuals, misused to achieve sedation, dissociation, or euphoria [11,12]. Of particular importance is the finding on the effects of benzodiazepines or gabapentinoids which, when taken in combination with prescription opioids, could cause dangerous respiratory depression resulting in mortality [13]. Prison custody settings, whether prisons or judicial psychiatric hospitals are reported to be at high risk for the misuse of psychoactive drugs, estimating their use in 30 percent of male prisoners and 51 percent of female prisoners [14,15]. The consequences of this serious public health problem range from increased admissions to treatment and emergency rooms to increased addiction and overdose deaths [16]. The ease of obtaining them compared to illicit drugs and the ability to avoid controls could

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explain the prevalence of prescription drug abuse in prison settings [17]. The misuse of psychoactive drugs in prisons also worsens prisoners' well-being: in fact, it is associated with bullying, violence, organized crime and indebtedness [18], suicide and self-harm [19]. The undetectability by most traditional drug tests, wide availability compared to traditional illicit drugs, and greater affordability being the reasons behind the misuse of psychoactive drugs [20]. So the international literature is becoming increasingly interested in this issue [17], which has become relevant especially in recent years [21]. Measures that aim to detect, assess, understand, and hopefully prevent adverse effects or any other drug-related problems come under pharmacovigilance. There is increasing attention to date on prescription drugs and their levels of dependence/potential for diversion [22–25]. Because the intended and actual use of drugs differ between clinical trials and actual use, pharmacovigilance activities focus on the post-marketing phase. In Europe, these activities are coordinated by the European Medicines Agency (EMA) [26] through EudraVigilance (EV), which is the system for collecting, managing, and analyzing information on suspected adverse reactions to drugs authorized in the European Economic Area (EEA) [26]. Therefore, prescribing can be very challenging because of the complex health needs of inmates and the risks to the prison population associated with the abuse and diversion of prescribed drugs and other illicit substances [21,27–29]. Despite widespread concern about prescription drug diversion in prisons, few studies have examined trends in prison drug misuse [17]. This study is a first step toward identifying the phenomenon of psychoactive drug misuse in prisons, exploring the classes of drugs commonly used, the characteristics of inmates who are affected, and focusing on studies that have identified the phenomenon in an evidence-based manner.

METHODS

Study design

A systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses [30]. This work was recorded on PROSPERO, International prospective register of systematic reviews (ID: CRD42024530273).

Search strategy

An electronic search in Medline, Scopus and Cochrane Library (Wiley) databases was performed from January 01, 2025 to June 01, 2025, looking for relevant studies that could be included in this study. The search was performed by setting the following terms: "misuse" OR "problematic drugs use [MeSH terms]" OR "substance misuse [MeSH terms]" AND "prison" OR "inmates" OR "custodial setting [MeSH terms]" OR "prison medicine [MeSH terms]". The Boolean operator "AND" was used to combine parts of the subject terms and "OR" was used to expand the search. Two independent reviewers (SM and MCN) screened titles and abstracts, assessed full-text versions, and extracted data. Disagreements were resolved by re-extraction or third-party adjudication. Where overlapping registries were identified or suspected, the more recent or informative study was included for analysis.

Data Extraction

The literature search was performed by two independent reviewers (SM and MCN) using a predefined search strategy. Duplicate studies were removed manually. Each reviewer then examined the titles, abstracts and/or full texts of included manuscripts to ensure that all inclusion criteria were met before extracting the following data: [1] first author's name, [2] year of publication, [3] study design, [4] country of origin, [5] purpose of the study, [6] nature and size of the sample, [7] inmates' characteristics, [8] number of inmates who did develop drugs misuse, [9] psychiatric comorbidities associated with drugs misuse, [10] addictive behavior associated with drugs misuse, [11] inmates' factors associated with drugs misuse [12]. Collected inmates' factors, were age, gender, employment, marital status, university degree, and type of drugs misuse.

Study selection

We defined our study eligibility using the populations-interventions-comparators-outcomes study design (PICO) framework. The PICO was defined as follows: the included population consisted of inmates, ≥ 18 years old who have been treated with psychoactive drugs and who have been diagnosed with drugs misuse. Outcomes of interest included inmates' characteristics and different types of drugs misuse. The primary outcome of the present study was to estimate the rate of drugs misuse, defined as misuse or nonmedical use of psychoactive prescription drugs, in custodial setting. Secondary outcomes included addictive behaviors, psychiatric comorbidities and inmate factors associated with drugs misuse. Primary outcome was defined at the time of the first studies' selection, while secondary outcomes were included following title and abstract review in order to capture a complete and accurate representation of the patient and surgical characteristics that have been evaluated by current literature. We included studies evaluating the impact of psychoactive drugs prescription on the development of drugs misuse, on inmates' ≥18 years of age, which enrolled more than 10 adults, with no limits of language. Studies meeting any of the following exclusion criteria were excluded from the

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present review: [1] comments, [2] animal studies, [3] abstracts, [4] review articles, [5] case reports or case series including less than 10 subjects; [6] editorials or letters, [7] studies not evaluating the impact of psychoactive drugs prescription on drugs misuse; [8] patient age < 18.

Risk of bias assessment

The Risk of Bias In Non-randomized Studies of Interventions (ROBINS-I) tool was used to rate risk of bias for non-randomized included studies [31]. This tool assesses seven domains: risk of bias from confounding, selection of participants, classification of interventions, deviations from intended interventions, missing data, measurement of outcomes, and selection of the reported results [31]. A proposed judgment about the risk of bias arising from each domain is generated by an algorithm, based on answers to the signaling questions. Judgment can be "Low", "Moderate" or "High" risk of bias, or can express "Some concerns" [31].

Data analysis and data synthesis

Patients' characteristics and outcomes were summarized and described as means or medians for continuous variables or percentages for categorical variables. Quantitative data synthesis was conducted while always keeping the drug abuse rate as the baseline outcome, in terms of the proportion of inmates manifesting drug misuse in prison. Pooled proportions were estimated through a proportional meta-analysis with random-effects models according to DerSimonian and Laird [32]. Results were expressed in proportions with 95% confidence intervals and prediction intervals. In cases of significant heterogeneity, prediction intervals will be wider than confidence intervals, offering a more cautious approach to integrating uncertainty into the analysis [33]. Heterogeneity was inspected using the I2 statistic, with a threshold level for significant heterogeneity of 50% [34]. Methods for assessing publication bias, such as Egger's and Begg's tests alongside funnel plots, were originally designed for comparative data, assuming a bias toward publishing positive results over negative ones. While it is possible to apply these tests to proportional meta-analyses, there is insufficient evidence to suggest they effectively account for such data. Additionally, the assumption of positive results being preferentially published may not hold true for proportional studies, given the absence of a standardized definition or consensus regarding positive outcomes in meta-analyses of proportions [33]. Statistical analyses were conducted adopting the R statistical software (version 4.4.0) [35]. Particularly, for the meta-analysis of proportions, the "meta" package (version 5.0.0) was employed. Statistical significance was determined at a threshold of two-sided p-values < 0.05.

RESULTS

Study selection

A total of 8826 studies were retrieved, and 7035 unique results remained for the initial title and abstract screening. Results were screened and 519 manuscripts underwent full-text review. Finally, only 6 articles met full inclusion criteria (Figure 1). Studies included 2 survey, 2 prospective cohort studies and 1 observational study (Table 1). All studies were conducted in Europe [36–40], except one in the US [41], and they presented a study period that collectively extended from 2006 to 2020. The study purposes of the included articles are presented in Table 1.

Risk of bias assessment

By using the Risk of Bias in Non-randomized Studies of Interventions (ROBINS-I), there was low-to-moderate risk of bias among the included studies. Overall, 4 of the included studies had low risk of bias (80%) [37,38,40,41], while just 2 of the studies included illustrated some concerns for bias (20%) [36,39]. None of the included studies were concerned to have a high risk of bias. Risk of bias assessment using the ROBINS-I tool is demonstrated in Table 2.

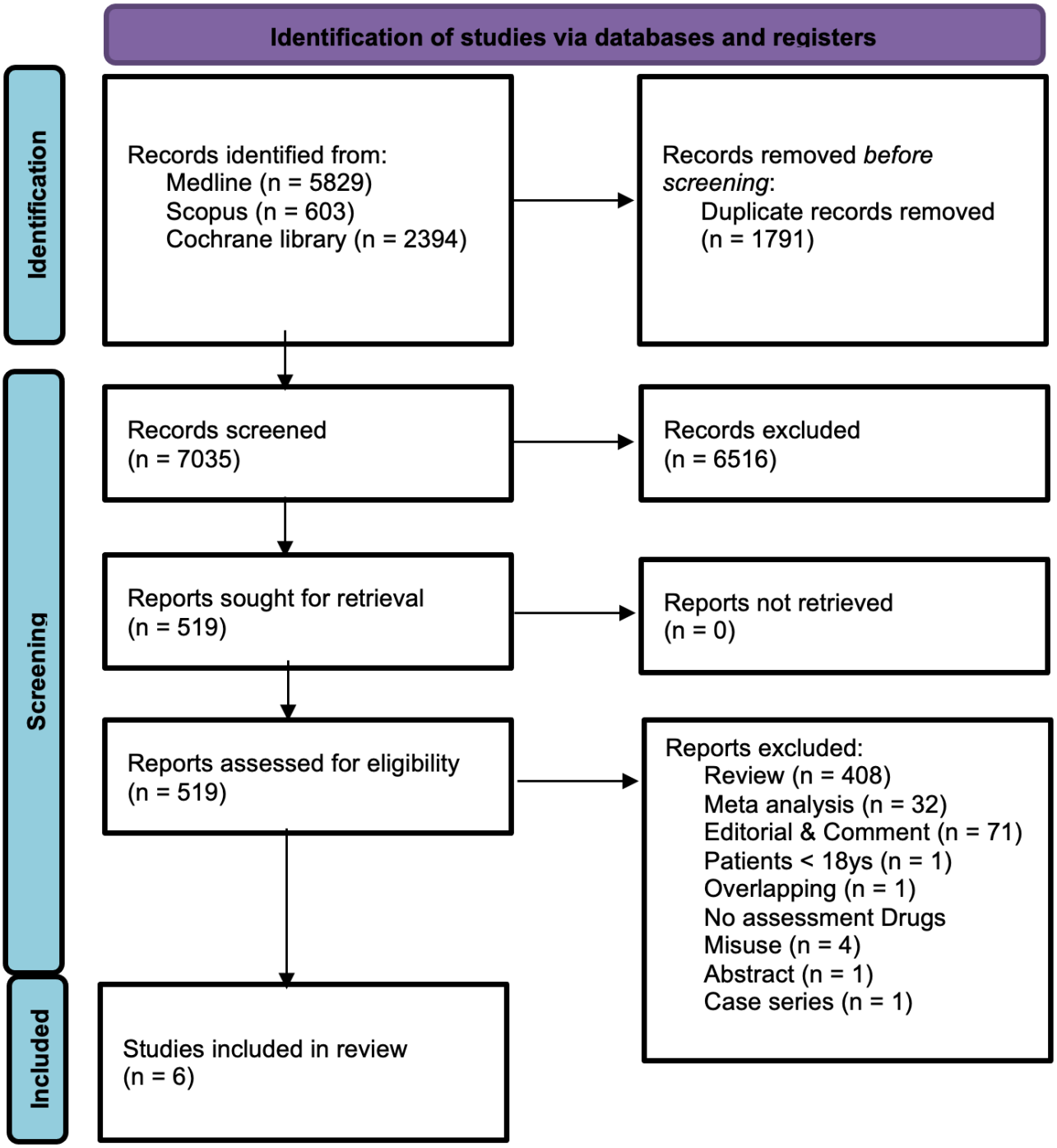
Inmates' characteristics and type of drugs misuse

Overall, 17023 inmates were included for analysis. Inmate' demographics from the included studies are displayed in Table 3. Female sex ranged from 0% to 33.8% in studies reporting sex. Age ranged from 18 to >50 years in the included studies and employed status ranged from 15.57 to 58.2%. Among the studies reporting the type of drugs misuse, 4 were focusing on opioids misuse [36–38,41], whereas 2 studies reported Gabapentinoids misuse and 1 Z-drugs misuse [39,40].

Rate of drugs misuse and predisposing factors

The prevalence rate of pooled drug misuse was 24% (95% CI: 35 44,2) (Fig. 2). The estimated 95% prediction intervals ranged from 35% to 44,2%. All the included studies evaluated the rate of drugs misuse following psychoactive drugs prescription [36–41]. The observational study by Plojovic et al [38]. reported a drugs misuse rate as high as 67.3% after psychoactive drugs prescription, which was consistent across other studies. The lowest reported rate of drugs misuse was 9.2% in the study by Sec et al. [37]. A prospective cross-sectional study by Durand et al. [40] determined rates of drugs misuse and demonstrated that the adjusted time trends across genders show prescribing rates were increasing for Gabapentinoids. The follow-up periods in the included studies are

Figure 1. Flowchart according to PRISMA guidelines



From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

For more information, visit: http://www.prisma-statement.org/

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Table 1. Design and characteristics of the included studies

References	Country	Inclusion period	Design	Purpose of the study	Nature and size of the sample
Sec, 2009	France	2006-2007	PCS	To identify which psychotropic medication are misused in custodial population	659 inmates
Plojovic, 2016	Serbia	2013	OBS	To investigate the misuse of psychologically active substances of convicts being in prisons and their treatment in the District Prison in Novi Pazar, Serbia	55 inmates
Soni, 2019	UK	2017-2018	Survey	To examine prescribing rates and the compliance for gabapentinoids in English Prisons	109 inmates
Franchetti, 2023	Germany	/	PCS	To provide an objective estimation of the prevalence of illicit use of methadone and buprenorphine in two German prisons	678 urine samples
Morrison, 2023	US	2015-2020	Survey	To explore patterns of opioids misuse among prison patients.	5154 individuals
Durand, 2023	Ireland	2012-2020	PCSS	To examine prescribing rates and trends for opioids, benzodiazepines, Z-drugs, and gabapentinoids in Irish Prisons between 2012 and 2020 using electronic health records data from the Irish Prison Services; to examine whether prescribing rates and trends vary by gender and if a person has a history of OUD; to determine rates of co-prescribing of opioids, benzodiazepines, Z-drugs, or gabapentinoids among people receipt of OAT medications.	10371 inmates

PCS: prospective cohort study
OBS: observational study
PCSS: prospective cross-sectional study
RCSS: retrospective cross-sectional study

Table 2. Methodological quality evaluation of the included non-randomized studies according to ROBINS-I

Author	Bias due to confounding domains relevant to the setting of the study	Bias in selection of participants into the study	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported results
Plojovic	Low	Moderate	Low	Low	Moderate	Low	Moderate
Soni	Low	Moderate	Moderate	Low	High	Moderate	Moderate
Franchetti	Low	Moderate	Low	Low	High	Moderate	Moderate
Morrison	Low	Moderate	Low	Low	Moderate	Moderate	Moderate
Durand	Low	Low	Low	Low	Moderate	Moderate	Low
Sec	Low	Low	Low	Low	Moderate	Moderate	Low

Table 3. Inmates' characteristics and type of drug misuse

Author	Age	Female sex, %	Employed, %	Not Married, %	University Education, %	Type of drug misuse
Sec	35.4*	9.8	26.3	NA	NA	Opioids, Z-drugs
Plojovic	20-35 range	0	58.2	56.4	1.8	Opioids
Soni	NA	NA	NA	NA	NA	Gabapentinoids
Franchetti	NA	NA	NA	NA	NA	Opioids
Morrison	#19.16* (18-29ys); 16.28* (30-40ys); 15.30* (>50ys)	18.42#	15.57#	18.72#	8.62#	Opioids
Durand	34.4*	33.8	NA	NA	NA	Gabapentinoids

**mean value*
 # Inmates with Drug Misuse
NA: not assessed

Table 4. Drugs Misuse rates and associated factors

Author	Inmates included n.	Inmates developing DM n. (%; 95%CI)	Follow-up period (%)	Inmates' social factors associated with DM	Addictive behavior associated with DM	Psychiatric factors associated with DM
Sec	659	61 (9.2)	NA	Drug selling; theft	Alcohol use	NA
Plojovic	55	37 (67.3)	1 year	Significant problems with the law	Alcohol use; Cannabis use; Cocaine use; LSD use	Serious or Mild problems with Mental Status
Soni	109	14 (13) diverting of prescribed drugs	8 month	NA	NA	NA
Franchetti	675	100 samples (14.8)	1 years	NA	NA	NA
Morrison	5154	919 (17.12; 15.22-19.21)	1 years	Arrest; drug selling; higher risk propensity	Tobacco use; marijuana use; cocaine use; binge drinking	Major depressive episode; serious psychological distress
Durand	10371	Adjusted time trends across genders show prescribing rates were increasing for Gabapentinoids (ARR [95% CI] 1.07 [1.05–1.08]).	1 year	NA	NA	NA
Tot	17023		/	/	/	/

NA: not assessed
DM: drugs misuse

reported in Table 4. Three studies provided insight into secondary outcomes evaluating inmates and addictive behaviors associated with drugs misuse [37,38,41]. Two studies demonstrated an association between drugs misuse and mental health problems in adults in custodial setting as reported in Table 4 [38,41]. Inmate characteristics associated with drugs misuse included arrest, drug selling, theft, higher risk propensity and generally significant problems with the law (Table 4). Psychiatric comorbidities and addictive behaviors

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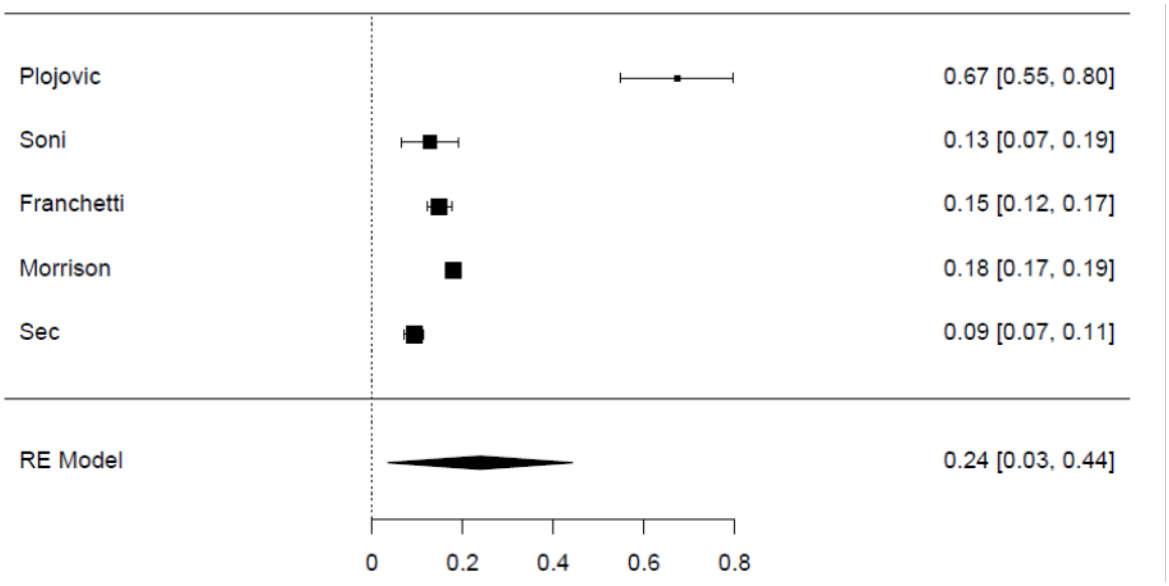
linked with drugs misuse included major depressive episode, serious psychological distress,

DISCUSSION

The present study demonstrates an incidence of drugs misuse as high as 24% as shown in Figure 2.

This finding is important as it highlights the need of identifying inmates at high risk of developing drugs misuse, as they may develop even lethal complications due to drugs abuse. Second, the present study identifies some factors that predispose to drugs misuse in custodial settings. Some of them are not modifiable (e.g., level of education, age) but can allow us to select categories at higher risk, which could benefit from multidisciplinary management (e.g., psychiatric, psychosocial specialists) after imprisonment in order to minimize the occurrence of drugs misuse. Others (e.g., psychiatric illnesses, addiction behaviors) may be identified at prison entry and treated/stabilized immediately and reevaluated frequently. Drug prevention work in prisons is critically important. Recent research points out that adult prisoners often continue to use psychologically active substances and commit criminal acts because they lack effective treatment and supervision [42,43]. Psychologically active substance abuse is a significant factor reflecting criminal behavior, and therefore treatment of the disease of addiction plays an important role in the prevalence of recidivism [38]. The effectiveness of treatment is mainly reflected in improved psychological interventions [44]. A combined treatment is possible in prisons and some authors evaluate that a treatment of addiction disease in combination with methadone therapy, counseling and treatment reduces the use of psychologically active substances within convicts that can go under such treatment, different from those who did not have any treatment [45]. Given that illicit use of methadone and buprenorphine is highly prevalent in the prison population, and that buprenorphine has been found to originate from the community setting, we believe that the methods currently implemented to prevent and counter illicit drug entry from the outside and inadvertent use of prescribed drugs in prison (e.g., inspection by prison officers or drug-sniffing dogs on visitors and inmates, and random urine screening on inmates) should be increased and expanded. This could be achieved by identifying those involved in the black market for substances inside and outside prison, or by prescribing forms of drugs less suitable for illicit use (e.g., buprenorphine-naloxone). In addition, our results indicate a high number of inmates with an untreated opioid use disorder. This underscores the need for widespread implementation of treatment of substance abuse in correctional institutions. In addition, the use of illicitly obtained opioids while in prison carries a higher risk of overdose [27]. Therefore, for people who die during detention or shortly after release, postmortem examination should routinely include systematic toxicological testing. Regarding the other pharmaceutical class affected by "drug misuse," gabapentinoids (pregabalin and gabapentin), they are increasingly being reported for drug misuse at the European level, in parallel with increasing prescription levels, related deaths, and black market [11,46–48]. Gabapentinoids are anticonvulsants, but they are also prescribed for a range of clinical conditions in neurology, psychiatry, and rheumatology, as well as being used off-label to treat benzodiazepine and alcohol dependence [20]. Their effects are due to binding to calcium channels, resulting in reduced levels of central excitability [20]. In addition, gabapentinoids are believed to possess GABA-mimetic properties, with possible direct/indirect effects on the dopaminergic "reward" system [22]. Typical psychoactive effects include a sense of well-being/relaxation, euphoria, and

Figure 2. Meta-analysis



even hallucinations [49]. The data that emerged in our study in this regard confirm those in the literature on the general population that gabapentinoids are increasingly abused or misused for self-medication, and it is also necessary to pay particular attention in the prison setting to the fact that these drugs can produce desirable effects on their own but are often used in conjunction with other drugs with unfavorable health outcomes. Therefore, multidisciplinary and multi-professional assessment remains critical in relation to the development of psychoactive drug misuse, to select which inmates are at risk and how to take action to reduce their risk of developing psychoactive drug misuse.

Strengths and Limitations

The main limitation of this study is the nature and scarcity of the included studies. Many were survey studies with risk of selection bias and underreporting. By its nature, the pooling of information from multiple studies has limitations due to the significant variability in patient populations and study designs, as well as different psychiatric assessment methods, thus a meta-analysis was not feasible. Instead, a pooled proportion approach was used. Several type of drugs misuse was included in this study, increasing the heterogeneity of the patient population. Prospective studies had limited size and numbers and were at risk for inclusion of nonconsecutive patients and nonresponse bias. Self-reported questionnaires were subject to recall bias or inaccurate responses regarding drug misuse. The majority of the included studies had chronic opioid use as their focus while there were scarce data on drugs misuse from other problematic drug misuse. Furthermore, the precise diagnosis of the type of associated mental disorder, which would be useful to further describe the impact of the development of drug misuse in these prisoners, is rarely specified in the included studies. Despite these limitations, this is the first systematic review to examine the phenomenon of psychoactive drug misuse in the prison setting. Another systematic review recently published in the literature addressed the topic of drug use within prisons, but unlike our study, which addressed the topic in a broader public health manner, the systematic review by Chiappini et all. [50], focused more in the area of clinical neurological-psychiatric symptomatology reported by inmate users. In addiction, the systematic review mentioned above, included articles that did not exactly center the context of the topic, for example, they dealt with drug misuse in the general population and not in the prison setting. In other cases, it added articles that used wastewater analysis as a methodology, a use that only allows for drug detection and not for discerning between prescription-induced use and psychoactive drug misuse, invalidating the validity of the conclusions reached. On the contrary, our work included only papers in the literature that addressed the issue of prison misuse of psychoactive drugs with reliable methodology with respect to the conclusions of identifying the phenomenon. This study is notably strengthened by its meticulous method, which involved the thorough screening of articles by clinical experts in emergency medicine during the literature review phase. Through rigorous appraisal, where each study quality was evaluated, and thorough assessment, involving rigorous data synthesis and analysis, we ensured a robust foundation to support the meta-analysis. Finally, our work is the only work that provided a meta-analysis with a prevalence data on the issue.

Further Issues

Illicit drug use was widespread in the surveyed prison population. Although the participation rate was high, this figure may still be underestimated. Further cross-sectional experimental studies that provide data on the prevalence of illicit drug use in prisons are needed to explore trends in this phenomenon and put in place appropriate measures to counter it, both at the level of public health interventions and ministerial measures. What emerges from this work suggests that future research should focus, in particular, on toxicological analyses of biological samples that allow longer detectability of drug use (e.g., hair). Furthermore, given the imbalance in the gender ratio observed in prisons, it is critical to perform gender-sensitive analyses, as women-specific findings would otherwise remain invisible. In addition, more in-depth analyses should be done with respect to poly-drug use, a type of intake that is potentially lethal to the abuser. As already indicated by a recent review of the literature on the subject [50], the increase in drug misuse in detention settings urgently requires more attention from public health and governments. New research is needed, such as understanding the long-term effects of new psychoactive substances on human health, and preventive strategies, such as figuring out how to enable better risk management to improve early warning systems for law enforcement and policy makers [50]. Prevention strategies should include not only training health personnel and educating prisoners, but also implementing stricter substance control policies and regulations [50]. Interventions such as the implementation of new prescribing guidelines involving substances with reduced abuse potential and diversion rates should be considered [50].

CONCLUSION

It is evident that the misuse and abuse of prescription drugs is a problem that affects the entire prison population, not just those with mental disorders, which, moreover, have not been extensively described as previously mentioned. Therefore, it is important not to consider drug misuse and abuse merely as a mental health issue, but rather to promote a dialogue at the intersection of these two distinct realities. This

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would enable the development and implementation of joint interventions by mental health services and addiction services for individuals facing these challenges. Such shared pathways should be based on a logic of empowerment and aim at promoting health, while avoiding the risk of neo-institutionalization in mental health and the use of the penal system to manage social phenomena. The misuse and abuse of prescription drugs in the prison setting is a global problem that requires urgent action. The modern pharmacovigilance, in order to look at how medicines are actually used in real life, should identify a range of technical tools and approaches to go beyond spontaneous reporting systems. Physicians should be vigilant when prescribing drugs with an abuse/misuse/diversion potential and carefully evaluate the possibility for inmates to be more vulnerable to these misuse activities. To effectively address these problems, prison institutions, health care providers, and policy must work together to implement preventive measures, provide appropriate treatment and support, and improve monitoring and reporting systems. It is imperative to recognize the seriousness of this problem and take concrete steps to address it comprehensively, starting with a methodological approach to develop research in the context of vulnerable people.

AUTHOR CONTRIBUTIONS

Silvia Martinelli, Mario Cesare Nurchis, Kıvanç Kok: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; and Writing - review & editing. Emanuele Caroppo, Gianfranco Damiani: Investigation; Methodology; Software; Validation; Visualization; Writing - review & editing (should always state something when more than one author).

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STATEMENT OF ETHICS

An ethics statement is not applicable because this study is based exclusively on published literature.

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DISCLOSURE STATEMENT

The authors report no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data used in the preparation of this manuscript is derived from published materials and can be accessed via literature search as outlined in methods and supplemental material.

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SUPPLEMENTAL MATERIAL

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Page 1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Page 1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Pages 2-3
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Page 3
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Pages 4
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Page 4
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Pages 4
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Pages 5
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Pages 5
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Page 5
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Page 5
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Page 6
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Page 6

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Section and Topic	Item #	Checklist item	Location where item is reported
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Page 6-7
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Page 6-7
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Page 6-7
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Page 6-7
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Page 6-7
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Page 6-7
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Page 6-7
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Page 6-7
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Page 7
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Page 7
Study characteristics	17	Cite each included study and present its characteristics.	Page 7-8 Table 1
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Page 7 Table 2
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Page 8-9 Figure 2
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Page 8-9 Table 3-4
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Page 8-9 Figure 2
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Page 8-9 Figure 2
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Page 8-9 Figure 2
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Page 8-9 Figure 2
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Page 8-9

Section and Topic	Item #	Checklist item	Location where item is reported
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Page 9-10-11
	23b	Discuss any limitations of the evidence included in the review.	Pages 11-12
	23c	Discuss any limitations of the review processes used.	Pages 11-12
	23d	Discuss implications of the results for practice, policy, and future research.	Page 12-13
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Page 4
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Page 4
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Page 4
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Page 14
Competing interests	26	Declare any competing interests of review authors.	Page 14
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Pages 4-5

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

For more information, visit: http://www.prisma-statement.org/

Heterogeneity Statistics

Tau	Tau²	I²	H²	R²	df	Q	p
0.230	0.0529 (SE = 0.0381)	99.67%	302.652	.	4.000	114.416	< .001

Random-Effects Model (k = 5)

	Estimate	se	Z	p	CI Lower Bound	CI Upper Bound
Intercept	0.238	0.104	2.30	0.022	0.035	0.442
	.	.	.	.	.	.

Note. Tau² Estimator: Restricted Maximum-Likelihood