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The comet assay as a tool in human biomonitoring exposure to antineoplastic drugs – A systematic review and meta-analysis

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ABSTRACT

Antineoplastic agents are toxic compounds, generally used in the treatment of cancers, which are recognized as carrying a cancer development risk. In this systematic review and meta-analysis of human biomonitoring studies, we have assessed the effects of exposure to antineoplastic drugs on levels of DNA strand breaks in leukocytes, measured by the comet assay. Focusing on the application of the comet assay in human biomonitoring of occupational exposure to antineoplastic agents, we have analyzed 458 original research studies which used this assay, following the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA-ScR). The systematic review led to 23 studies, of which 20 studies met the criteria for inclusion in the meta-analysis. Using standardized mean difference and 95% confidence interval (CI), the meta-analyses show increased levels of DNA strand breaks in subjects exposed to antineoplastic drugs (1.26, 95% CI: 0.78, 1.73). Results originate mainly from studies on healthcare workers, with only one study in an industrial setting. Subgroup analysis indicates that all studies combined from middle-income countries have a higher effect size (1.77, 95% CI: 1.00, 2.55) than studies from high-income countries (0.49, 95% CI: 0.09, 0.90). This difference between middle- and high-income countries may be attributable in part to differences in exposure levels or exposure assessment. Additionally, sensitivity analysis indicates that studies with moderate/high risk of comet assay measurement bias have higher effect size (2.07, 95% CI: 0.82, 3.31) than studies with low risk of bias (0.73, 95% CI: 0.34, 1.13); and that studies with high risk of exposure misclassification have higher effect size (1.47, 95% CI: 0.89, 2.06) than studies with low/moderate risk (0.13, 955 CI: −0.08, 0.33). Most studies have low/moderate risk of bias related to the comet assay procedure (15 out of 20 studies), absence of reporting the use of assay controls (1 out of 20 studies), blinded analysis of samples (7 out 20 studies); exposure assessment (16 out of 20 studies). In conclusion, this systematic review and meta-analysis shows that exposure to antineoplastic drugs is associated with increased levels of DNA strand breaks in human leukocytes.

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1. Introduction

Promoting the use of human biomonitoring (HBM) and its establishment is a recognized priority for ensuring chemical safety both globally and in the World Health Organization (WHO) European Region, as set out by the World Health Assembly (Resolution 76.17 on the impact of chemicals, waste and pollution on human health) and the European Environment and Health Process (EHP) [1].

HBM is a scientifically developed approach for assessing human exposures to natural and synthetic compounds from environment, occupation, and lifestyle. It relies on the measurement of particular compounds or their metabolites, in human tissues and/or fluids, and also includes the study of their effects and the possible influence of individual susceptibility as response modulators [2]. HBM is an increasingly popular approach applied to exposure measurement, hazard characterization and risk assessment in environmental and occupational health, and its importance has been increasing as a result of advances in the ability to measure greater numbers of chemicals in the human body and tissues. To achieve this purpose, HBM focuses on the use of biomarkers as measurable indicators of early changes in biological systems [2,3].

The comet assay is widely used in HBM for assessing DNA damage [4,5], associated with both occupational and environmental exposures (reviewed in [6]. It identifies different types of DNA damage, such as single and double-strand breaks, alkali-labile lesions converted to strand breaks under alkaline conditions, and single-strand breaks associated with incomplete excision repair sites [7,8], resulting from recent exposure. The high sensitivity of the comet assay allows the detection of subclinical DNA damage that may precede overt disease, supporting its role as an early-effect biomarker. Furthermore, the use of lesion-specific enzymes, such as formamidopyrimidine DNA glycosylase (Fpg) or endonuclease III (Endo III), enhances the biological relevance of the assay by enabling the detection of DNA base oxidation, which is particularly relevant in exposures associated with oxidative stress. These characteristics make the comet assay a valuable component of integrated biomonitoring strategies and human health risk assessment [5,9].

A major strength of the comet assay in biomonitoring studies is the possibility of using easily accessible human cells, particularly peripheral blood lymphocytes and exfoliated epithelial cells (e.g. buccal or nasal cells). This enables minimally invasive sampling, repeated measurements over time, and ethical feasibility in population-based studies. The technique requires small number of cells per sample, is sensitive, versatile and relatively quick [10–12]. Protocols for various comet assay modifications and applications, such as to different cell types, have been compiled [8], along with recommendations for reporting comet assay methods and results [13].

Despite these advantages, several limitations constrain the interpretation and regulatory applicability of comet assay data in human biomonitoring. One of the most important challenges is the substantial interindividual variability in DNA damage levels. Factors such as age, sex, nutritional status, lifestyle habits (e.g. smoking, alcohol consumption, physical activity), health conditions, and genetic polymorphisms in xenobiotic-metabolizing enzymes and DNA repair pathways can significantly influence comet assay outcomes [4,5,14]. This biological variability complicates the attribution of observed DNA damage to a specific occupational or environmental exposure and necessitates careful study design and statistical control of confounding factors.

A key characteristic of the comet assay is that it reflects mainly recent or ongoing exposure, as the DNA damage incurred is largely repairable by cellular repair mechanisms and so is transient - a potential limitation of the comet assay as a biomarker assay. The assays can only capture historical or cumulative exposures if longitudinal designs with repeated sampling are employed. In addition, the assay does not provide direct information on mutation fixation or long-term health outcomes, such as cancer risk, and therefore cannot be used in isolation to predict disease development [15].

From a methodological perspective, variability in protocols and scoring approaches remains an issue in biomonitoring studies, potentially affecting comparability between investigations [8,16,17]. Although international efforts have led to improved standardization, including the publication of guidelines and validation exercises, the lack of universally accepted reference values or biological effect limits the use of comet assay results for regulatory decision-making [9,15]. As a result, comet assay data are generally interpreted in a comparative manner (exposed versus control groups) rather than against fixed thresholds.

Overall, the comet assay should be regarded as a complementary biomonitoring tool, best applied within a battery of biomarkers encompassing exposure, effect, and susceptibility. When integrated with other genotoxicity endpoints, exposure assessment data, and epidemiological information, the comet assay provides valuable insight into DNA damage as a putative precursor of mutation and contributes meaningfully to the evaluation of occupational and environmental health risks.

Antineoplastic drugs, also known as cytotoxic or cytostatic drugs, are a heterogeneous group of chemicals that share an ability to inhibit tumour growth by disrupting cell division and killing actively growing cells [18,19]. Paradoxically, most of them exert their therapeutic effects through direct or indirect interactions with DNA, which also explains their well-documented genotoxic and mutagenic properties. Although patients may benefit from these treatments, there is still a major health concern regarding the use of some drugs classified as carcinogenic, mutagenic or teratogenic agents [20].

In accordance with the International Agency for Research on Cancer (IARC), many antineoplastic compounds are classified as known human carcinogens (Group 1; cyclophosphamide, etoposide, busulfan), probably carcinogenic (Group 2 A; azacitidine, cisplatin, teniposide) or possibly carcinogenic (Group 2B; dacarbazine, bleomycin, daunorubicin); other compounds show no evidence of carcinogenicity (Group 3; vinblastine, methotrexate, 5-fluorouracil) [21,22].

Modes of action (MOA) differ according to the type of antineoplastic drug. Cyclophosphamide and melphalan are alkylating agents, acting by covalently binding to DNA, leading to base modifications, intra- and inter-strand cross-links, and strand breaks which interfere with replication and transcription [23,24]. Antimetabolites disrupt nucleotide biosynthesis or are incorporated into nucleic acids, resulting in replication stress and secondary DNA damage; 5-fluorouracil and methotrexate are widely used examples [24]. Topoisomerase inhibitors, such as etoposide and irinotecan, interfere with the normal function of topoisomerase I or II by stabilising DNA–enzyme cleavage complexes, thereby preventing DNA re-ligation and causing the accumulation of single- and double-strand breaks, which are strongly associated with chromosomal instability [24]. Other agents, including anthracyclines, intercalate into DNA and generate reactive oxygen species, contributing to DNA oxidation damage and further strand breakage. Microtubule-targeting drugs, while not directly damaging DNA, disrupt mitotic spindle formation and chromosome segregation, leading to aneuploidy and genomic instability [24]. Collectively, the diversity of MOA of antineoplastic drugs explains both their clinical efficacy and their potential to induce DNA damage, highlighting the relevance of these agents as occupational hazards and the importance of effective exposure control measures in healthcare settings.

Occupational exposure to antineoplastic drugs may occur in a variety of work settings throughout the healthcare system and across multiple stages of the drug life cycle. However, the highest risk, as well as most of the documented studies, are associated with hospital pharmacies and oncology units, where these agents are routinely prepared, reconstituted, and administered. Hospital workers, such as pharmacists and pharmacy technicians, manipulate antineoplastics during drug preparation, particularly when performed manually; workers may be exposed through inhalation of aerosols, dermal contact with contaminated surfaces, or accidental spills and splashes. Even when biological safety

cabinets are used, improper work practices, inadequate maintenance, or high workload pressures may increase the likelihood of exposure [25–31].

Nurses and other healthcare professionals involved in drug administration are also at significant risk, as exposure may occur during intravenous infusion, connection and disconnection of infusion systems, handling of syringes and tubing, and management of extravasation events. In addition, contact with patients' urine, faeces, vomit, and sweat, represents an important secondary exposure route, as many antineoplastic drugs and their active metabolites are excreted unchanged for several hours or days after treatment [32–35].

It is important to note that occupational exposure is not limited to clinical staff. Cleaning and housekeeping personnel may be exposed when handling contaminated waste, cleaning drug preparation areas, patient rooms, toilets, and medical equipment. Similarly, waste handlers and laundry workers may come into contact with contaminated materials, including bedding, gowns, and disposable protective equipment. Other forgotten occupational scenarios include transport and storage of antineoplastic drugs, where exposure may occur as a result of damaged vials, leaks, or inadequate packaging. Workers involved in drug manufacturing, packaging, and quality control may also experience exposure.

Evidence has shown that occupational exposure to antineoplastic drugs is associated with an increased risk of acute health effects - including hair loss, headaches, and hypersensitivity; adverse reproductive outcomes, such as infertility, spontaneous abortions and congenital malformations; and certain cancers [36–40]. Regarding cytogenetic biomarkers, several studies have shown associations between occupational exposure to antineoplastics and chromosomal aberrations [41–44], sister chromatid exchanges [44] and micronuclei [42–44].

There are already some systematic reviews [45], and meta-analysis studies [27,46] regarding the genotoxicity induced by occupational exposure to antineoplastic drugs. A previous systematic scoping review [6] was developed with the aim of gathering and analysing the available evidence on the use of the comet assay in human biomonitoring studies for the assessment of genotoxic effects from occupational and environmental exposure to chemicals substances. These were subsequently organized into six categories of exposure, of which antineoplastic agent exposure was one. Some of the studies showed that occupational exposure to antineoplastics induces genotoxic effects, measured by the comet assay [6,27,45].

The present systematic review and meta-analysis is part of a series of systematic reviews and meta-analysis on comet assay results from human biomonitoring studies in different exposure groups, namely heavy metals [47] pesticides [48], volatile organic compounds (VOCs) [49], combustion-derived air pollution [50], anaesthetic drugs (submitted for publication in Reviews in Mutation Research) and, antineoplastic agents (this review). The overall purpose of these systematic reviews is to compare the effects of exposures on DNA strand breaks in human leukocytes, measured by the comet assay.

In this paper we go further, carrying out a meta-analysis to quantitatively assess whether DNA damage measured by the comet assay can be a useful biomarker in human biomonitoring of occupational exposure to antineoplastic drugs. Specifically, this study aims to test the hypothesis that direct occupational exposure to antineoplastic agents may be associated with higher levels of DNA damage, measured by the comet assay.

2. Materials and methods

The systematic review was performed in accordance with Joanna Briggs Institute and Cochrane Collaboration recommendations [51–53] and is reported following the PRISMA-ScR (Preferred Reporting Items for Systematic Reviews and Meta-Analyses – extension for scoping reviews) checklists [54,55].

The protocol has been registered in PROSPERO, with the registry number CRD42024602906. At least two authors independently conducted all steps of the study selection and data extraction. Divergences were resolved by discussion in consensus working group meetings.

2.1. Search strategy and eligibility criteria

The research was carried out in the following electronic databases: PubMed (https://pubmed.ncbi.nlm.nih.gov/) and Web of Science (www.webofscience.com) without language limits. Searches were limited to human studies and by year of publication [from 2000, after the introduction of 'Comet Assay' Medical Subject Headings (MeSH) term]. A manual search in the reference lists of included studies was also performed, by using other search engines (Google and Google Scholar).

The search strategy was developed using descriptors (TS – Topic Search) and MeSH [MH] terms related to human biomonitoring, comet assay, and antineoplastic drugs with Boolean operators AND and OR. Specifically:

2.1.1. PubMed

("Environmental Exposure"[MH] OR "Human Biomonitoring"[TIAB] OR monitoring[TIAB]) AND ("Comet Assay"[MH] OR Comet[TIAB] OR "single cell gel electrophoresis"[TIAB]) AND ("Antineoplastic Agents"[MH] OR antineoplastic*[TIAB] OR antitumor*[TIAB] OR anticancer*[TIAB] OR cytostatic*[TIAB] OR cytotoxic*[TIAB] OR chemotherap*[TIAB] OR cyclophosphamide[TIAB] OR paclitaxel[TIAB] OR 5-Fluorouracil[TIAB])

2.1.2. Web of Science

TS=("human biomonitoring" OR monitoring) AND TS=("comet assay" OR "single cell gel electrophoresis") AND TS=(antineoplastic OR antitumor OR anticancer OR cytostatic* OR cytotoxic* OR chemotherap* OR cyclophosphamide OR paclitaxel OR 5-Fluorouracil)

Registers retrieved from the databases (PubMed and Web of Science) were transferred into Mendeley (reference manager) and Rayyan, where duplicate records were removed. Reviewers independently performed the screening (title/abstract reading, [TIAB]), full-text evaluation and data extraction using Microsoft Excel.

This systematic review and meta-analysis included articles meeting the following criteria (PECOS' acronym):

•Population: studies evaluating human subjects with occupational exposure to antineoplastic agents;

•Exposure: antineoplastic drugs occupational exposure, by means, medians, standard deviation, and interquartile range of comet assay in biological samples;

•Comparator: non-exposed (controls) human subjects or pre-post comparative data on exposure (i.e., single-arm study);

•Outcomes: comet assay measurements such as tail moment, tail length (μm), % DNA tail intensity, olive tail moment, visual scoring/DNA damage index parameters, and other relevant parameters;

•Study design: interventional studies (controlled trials, experimental studies) or observational comparative studies including case-control, cohort, cross-sectional studies, quasi-experimental studies (pre-post-test).

Studies without data for extraction (unavailable information or unpublished paper), conference abstracts, other study designs (reviews, meta-analysis, case reports, letters, commentaries, protocols), non-human studies (in vitro and in vivo), in vitro studies on primary human cells or cell lines, and those in a language other than English were excluded.

2.2. Data extraction and synthesis

A standardized form (Microsoft Excel) was developed and validated by all team members (co-authors) to extract data on: (1) authors, (2) year of publication, (3) type of antineoplastics agents, (4) country, (5) exposure assessment or biomarkers of exposure, (6) population

characteristics, and (7) DNA damage measured by comet assay. Data only available in figures were extracted, whenever possible, by the same team member.

The results of the retrieval and screening process are summarized in the flow diagram, the individual results of the studies are reported in a table, including type of measures and units (narrative synthesis), and quantitative analysis are presented as Forest and Funnel plots.

2.3. Review of studies

2.3.1. Generalizability

Certain studies have sufficient relevance to be included in the review, although the results are not applicable in meta-analysis. These include studies designed for correlation or regression analysis. In addition, results reported as geometric mean and standard deviation factor cannot be incorporated into a meta-analysis with results reported as mean and standard deviation from Gaussian distribution of results. The same goes for binary data or results analysed by logistic regression. Thus, results in the meta-analyses of the present review are a subgroup of all included studies. The generalizability of the results in the meta-analyses to all studies in the review has been tested as differences in proportions of statistically significant results in the original studies.

2.3.2. Meta-analysis

Some studies have looked at levels of DNA strand breaks in exposed subjects compared with a control group, whereas others have segregated the study population into different exposure groups. For the latter type of studies, the control group consists of subjects with lowest exposure regardless of the dataset being segregated into two or more exposure groups. We have dichotomized exposures in studies with more than two groups (i.e. lowest exposure group versus all other groups pooled).

In meta-analysis, for the calculation of standardized mean difference (SMD) as effect measure, we have only used results on tail length, tail intensity (also called percentage of DNA in the comet tail) and tail moment (i.e. product of tail length and tail intensity) from studies with image analysis of comets. Values of the same comet descriptor in different studies do not necessarily correspond to the same numbers of DNA strand breaks, because studies may not use the same comet assay protocol [56,57]. SMD is the difference between groups in standard deviation units. For graphical purposes, we have reproduced Forest plots from Review Manager in GraphPad Prism. Original Forest plots and accompanying Funnel plots from Review Manager are reported as Supplementary Figures S1 to S4**.**

We have calculated SMD and 95% CI in random effects models using Review Manager 5.4 (The Nordic Cochrane Centre, The Cochrane Collaboration). I^2 values are used as a measure of heterogeneity between studies (0–40% “might not be important”, 30–60% “may represent moderate heterogeneity”, 50–90% “may represent substantial heterogeneity”, and 75–100% “considerable heterogeneity” (https://training.cochrane.org/handbook).

Heterogeneity between studies may arise as a consequence of difference in biological effects between studies due to difference in e.g. age, sex and exposure situation. The latter might be due to different workplaces being more or less likely to implement/enforce protective procedures to avoid exposure of workers to hazardous compounds. It is virtually impossible to assess the magnitude of exposure, except by comparing concentrations of antineoplastic agents in biological samples in studies that have reported concentrations in the same unit. In addition, heterogeneity in effect sizes might be dependent on the researcher’s control of the variation that inevitablyoccurs in biological techniques such as the comet assay. This applies to both blinded scoring of samples and inclusion of samples with contrasting exposures in the same comet assay experiment (i.e. analysis of matched pairs of pre and post samples or exposed/controls in the same experiment). Randomized analysis of samples and matched pairs are acceptable analytical designs, but comparison of effect sizes between these experimental designs might cause heterogeneity in a meta-analysis.

Visual inspection of Forest and Funnel plots (Supplementary Figures S1 to S4) indicates that the distribution of SMDs in the present dataset is skewed to the right (i.e. the central tendency is influenced by studies with high effect size). In order to assess the robustness of effect sizes in the meta-analysis, we have also used non-parametric analysis to calculate the central tendencies. For this analysis, we have used the SMDs from each study for the calculation of the median and 95% CI. The rank number has been rounded down or up to the nearest whole number for the lower and upper 95% CI, respectively. These ranks have subsequently been converted to the absolute values. We refer to this outcome as SMD_{median} to distinguish the result form the regular SMD in meta-analysis.

2.3.2.1. Subgroup analyses. The studies have been stratified into populations from high-income and middle-income countries, according to the 2025 World Bank classification (https://data.worldbank.org/). In the present dataset, high-income economies include a number of European countries (Belgium, Croatia, Cyprus, Germany, Finland, France, Italy, Poland and Portugal) and Taiwan. Countries in the group of middle-income countries include Argentina, Bangladesh, Brazil, China, Columbia, India, Iran, India Mexico, Pakistan and Turkey.

2.3.2.2. Sensitivity analyses. Uncertainty (potential bias) related to comet assay experiments and exposure assessment has been assessed on a scale with low, moderate or high risk of bias or exposure misclassification. The classification system is described in detail in the supplementary material. In brief, comet assay experiments have been rated according to the “essential information” in the Minimum Information for Reporting on the Comet Assay (MIRCA) recommendations for assessing the information on comet assay procedures and results [13], inclusion of assay controls and blinded/coded analysis of samples or slides. The exposure assessment reflects, in increasing certainty of exposure: (i) grouping based on work categories or areas, (ii) environmental monitoring assessment, and (iii) biomarkers of exposure (internal and/or effective dose). For visual presentation, we have used traffic light plots where red, yellow and green colours refer to studies with high, moderate and low risk of bias/exposure misclassification. The traffic light plots have been generated in robvis (https://mcguinlu.shinyapps.io/robvis/) [58]. Differences in distributions have been tested by χ^2-test. Binomial exact tests have been used to assess the variability around proportions of studies with statistically results.

3. Results

3.1. Summary of search results

A summary of the literature search is presented in Fig. 1. After removal of duplicates, it was identified 458 articles, of which 424 were excluded after first screening (title/abstract). From the 32 articles read in full, 11 were excluded for the following reasons: did not present comet assay data (n = 2), did not present data appropriate to be used in the meta-analysis (n = 5), or were not in English (n = 4). Twenty-one studies with data on exposure to antineoplastic drugs and DNA strand breaks were included in the review [18, 19, 25, 28–30, 32, 33, 59–73]. Table 1 summarizes the included studies found in the systematic search.

Briefly, all the 23 studies were from occupational settings, one from a production plant [60] and the others (n = 22, 95.2%) in healthcare workers. Three studies [19,59,69] performed exposure assessment combined with biological monitoring, two studies had only exposure assessment [25,73], and another [64] only urinary biological monitoring, in a total of 5 (23.8%) studies compared with 16 (76.2%) that used solely the comparison with a reference group. More detailed information is found in a previous scoping systematic review [6] The majority of the studies (n = 15, 65.2%) reported using specifically

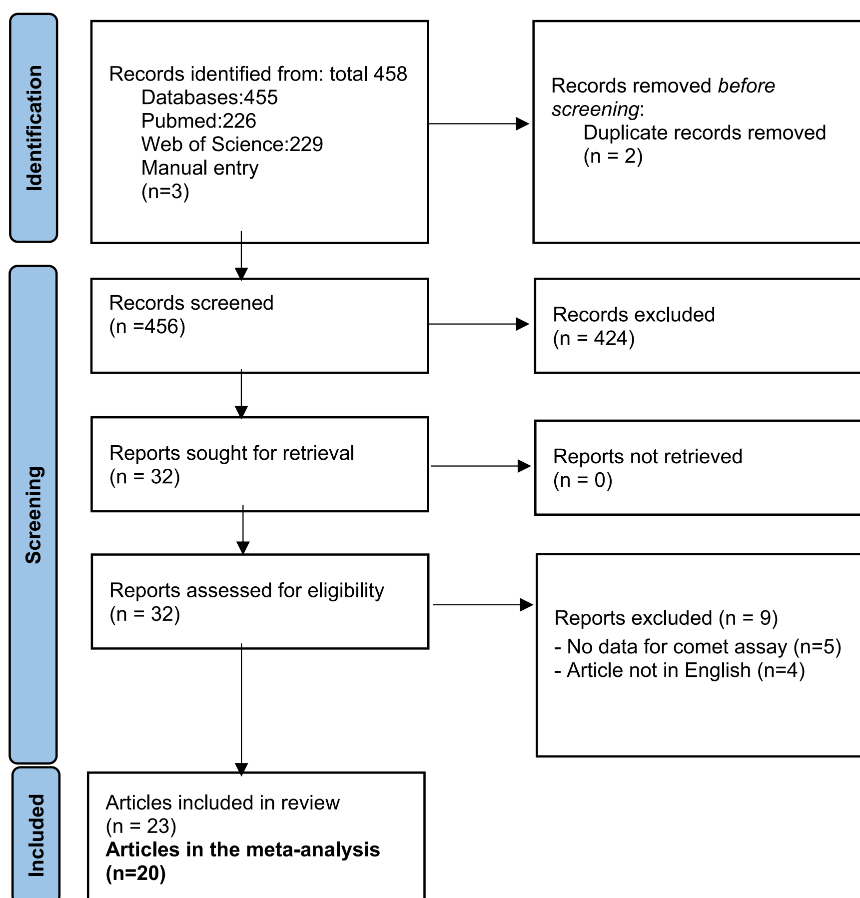

Fig. 1. PRISMA Flow diagram of systematic review and meta-analysis for antineoplastic drugs.

isolated peripheral blood lymphocytes, followed by the use of blood leukocytes/whole blood (n = 8, 34.8%).

3.2. Meta-analysis

Twenty studies have been included in the meta-analysis (Fig. 2). The meta-analysis shows a SMD of 1.26 (95% CI: 0.78, 1.73), which is skewed due to the heterogeneity in effect between studies. Using non-parametric test shows a slightly lower effect size (SMD = 1.01, 95% CI: 0.08, 1.95).

3.2.1. Generalizability

Among the included studies, there is an over representation of studies (13 out of 20) that show genotoxicity to antineoplastic agents [28,30,32,33,60–67,71,73] compared to 7 null effect studies [18,19,25, 29,59,68,69].

3.2.2. Subgroup analysis

Stratification of studies by region indicates that studies from middle-income countries have higher effect size (SMD = 1.77, 95% CI: 1.00, 2.55) than studies from high-income countries (SMD = 0.49, 95% CI: 0.09, 0.90).

3.2.3. Sensitivity analysis

Fig. 3 shows the results of assessment of risk of comet assay experiment bias and exposure misclassification. The overall results indicate that most studies have low/moderate risk of bias related to the comet assay procedure (15 out of 20 studies). However, only one study reported using assay controls. There are also relatively few studies that report blinded/coded analysis of samples (7 out of 20 studies). The exposure assessment also leaves room for misclassification (16 out of 20 studies).

Overall, only one study has low risk of comet assay measurement bias and exposure misclassification [19] whereas four other studies have moderate risk [18,25,28,61]. Interestingly, studies from high-income countries tend to have a higher proportion with overall low/moderate risk of comet assay measurement bias and exposure misclassification (80%, 4 out of 5 studies), compared to the proportion from middle-income countries (33%, 5 out of 15 studies; $\chi^2 = 3.3$, P = 0.07), as shown in Table 2**.**

The sensitivity analysis is slightly uncertain due to low numbers of observations in some groups. Therefore, we have pooled some groups to obtain more robust effect sizes. Tables 3 and 4 shows effect sizes in groups according to risk of comet assay measurement bias and exposure misclassification.

The most striking observations are that studies with moderate/high risk of comet assay measurement bias have higher effect size (2.07, 95% CI: 0.82, 3.31) than studies with low risk of bias (0.73, 95% CI: 0.34, 1.13); and that for the risk of exposure assessment, studies with high risk of exposure misclassification have higher effect size (1.47, 95% CI: 0.89, 2.06) than studies with low/moderate risk (0.13, 955 CI: −0.08, 0.33).

4. Discussion

Collectively, the meta-analysis of 20 studies shows a positive association between exposure to antineoplastic drugs and levels of DNA strand breaks in blood leukocytes, which corroborates the hypothesis that occupational exposure to antineoplastics increases DNA damage, measured by comet assay. All the studies are from healthcare occupational contexts, with the exception of one from a methotrexate production facility. Nurses, pharmacists and pharmacy technicians are the populations more studied, since they have a direct role in the

Table 1
Summary of findings from the included studies on antineoplastic drugs.

Author	Year	Main Chemical Exposure	Country	Exposure Assessment or Biomarkers of Exposure	Population Characteristics	DNA Damage	Reference
Aristizabal-Pachon et al.	2019	Antineoplastic drugs	Colombia	–	80 (40 exposed, 40 unexposed) Hospital workers (females and males, 32.05 ± 5.1 and 30.80 ± 5.5 years, respectively) Peripheral blood lymphocytes	• **TL:** exposed (4.62 ± 1.477 μm) vs. unexposed (2.41 ± 0.577); **Sig. (Mean and SD).**	[62]
Buschini et al.	2013	Antineoplastic drugs	Italy	–	137 (63 exposed, 74 unexposed), Nurses (females, non-smokers, 39 ± 8 years) Peripheral blood leukocytes	• **TI:** exposed (0.95 ± 0.03) vs. unexposed (0.99 ± 0.03); **Non-sig. (Mean and SEM).**	[18]
Cavallo et al.	2009	Antineoplastic drugs	Italy	–	106 (30 exposed, 76 unexposed), Hospital workers (females and males 35.17 ± 7.4 and 39.72 ± 10.1 years in exposed and controls, respectively) Peripheral blood lymphocytes	• **TM:** exposed (16.86 ± 9.13) vs. unexposed (16.72 ± 7.17). **Non-Sig. (Mean and SD).**	[68]
Connor et al.	2010	Antineoplastic drugs	USA	Fixed-location, and personal breathing zone air samples All area air sample concentrations were below the LOD (3.1–6.9 ng/m^3) for all 5 drugs. One personal air sample demonstrated a concentration of cyclophosphamide of 84.5 μg/m^3 All other personal air samples were below the LOD. Cyclophosphamide, ifosfamide, paclitaxel, 5-fluorouracil, and cytarabine surfaces contamination Surface concentrations for the five drugs ranged from below the LOD (0.07–0.10 ng/cm^2) up to 910 ng/cm^2. For all areas combined, the drug detected most often in the wipe samples was cyclophosphamide (43%), 5-fluorouracil (26%), ifosfamide (24%), paclitaxel (16%), and cytarabine (3%). Urinary cyclophosphamide and paclitaxel (6 positive samples out of 67 samples from exposed subjects, levels <LOD (0.015 ng/mL). for unexposed).	121 (68 exposed, 53 unexposed), Hospital workers (females and males, non-smokers, 38.5 ± 10.5 and 39.9 ± 10.4 years in exposed and controls, respectively) Peripheral blood leukocytes	• **TI:** exposed (53.06 ± 7.32) vs. unexposed (53.12 ± 7.5); **Non-sig. (Mean and SD).** • **OTM**: exposed (2.540 ± 652) vs. unexposed (2.518 ± 715); **Non-sig. (Mean and SD).** • Combined: exposed (1.00 ± 0.20) vs control (1 ± 0.21).	[59]
Cornetta et al.	2008	Antineoplastic drugs	Italy	-	90 (83 exposed and 73 unexposed) Hospital workers (females and males, smokers and non-smokers, 37 years (range 23–58 years) Peripheral blood leukocytes	• **TI:** exposed (1.16 ± 0.82) vs. unexposed (0.77 ± 0.47); **Sig. (Mean and SD).**	[61]
Deng et al.	2005	Methotrexate	China	–	42 (21 workers and 21 controls) Workshop producing MTX (females and males, smokers and non-smokers, exposed: mean age, 35 (19 – 50) years,	• **TL:** exposed (1.30 ± 0.06 μm) vs. unexposed (0.7 ± 0.01 μm); **Sig. (Mean and SEM).** • **TM:** exposed (0.23 ± 0.03 μm) vs. unexposed	[30]

(continued on next page)

Table 1 (*continued*)

Author	Year	Main Chemical Exposure	Country	Exposure Assessment or Biomarkers of Exposure	Population Characteristics	DNA Damage	Reference
					and controls: mean age 37 (21 – 55) years. Peripheral blood lymphocytes	(0.17 ± 0.04 µm); **Sig. (Mean and SEM).** • Combined: exposed (1.61 ± 0.60) vs control (1 ± 0.57).	
Fakher et al.	2020	Antineoplastic drugs	Egypt	–	48 (28 exposed, 20 unexposed female's controls) Females nurses (exposed: 39.8 ± 9.9 (range 25 – 53), controls: 38.7 ± 9.8 (range 25 – 52), years Peripheral blood leukocytes	• **TL:** exposed (8.69 ± 1.84 µm) vs. unexposed (2.06 ± 0.65 µm); **Sig. (Mean and SD).** • **TM:** exposed (1.62 ± 0.49 µm) vs. unexposed (0.38 ± 0.20 µm); **Sig. (Mean and SD).** • Combined: exposed (4.24 ± 1.09) vs control (1 ± 0.42).	[32]
Hongping et al.	2006	Vincristine	China	–	30 (15 exposed, 15 unexposed) Workers from a plant production (exposed: 6 males and 9 females from 36 to 52 years old (mean age, 43.67 ± 1.13 years old), the average ages of male workers and female workers are 44.17 ± 2.40 and 43.33 ± 1.14 years old, respectively; controls were matched with workers on the basis of age (from 36 to 55 years old, mean age, 43.47 ± 1.41 years old), gender and smoking). Peripheral blood lymphocytes	• **TL:** exposed (1.72 ± 0.15 µm) vs. unexposed (0.71 ± 0.01 µm); **Sig. (Mean and SEM).** • **TM:** exposed (0.29 ± 0.03 µm) vs. unexposed (0.17 ± 0.05 µm); **Sig. (mean and SEM).** • Combined: exposed (2.06 ± 0.59) vs control (1 ± 0.59.)	[60]
Izdes et al.	2009	Antineoplastic drugs (cyclophosphamide, cisplatin, 5 fluorouracil, etoposide, vinblastine, vincristine, bleomycine and doxorubicin)	Turkey	–	38 (19 exposed, 19 controls) Nurses (females and males, smokers and non-smokers, exposed: 32.3 ± 5.9 years, and controls: 33.5 ± 5.1 years. Peripheral blood lymphocytes	• **VS:** exposed 19.89 ± 4.84 vs. non-exposed: 6.84 ± 3.16. **Sig. (Mean and SD).**	[63]
Kopjar et al. *	2001	Antineoplastic drugs	Croatia	–	70 (50 exposed, 20 unexposed) Hospital workers (exposed: females smokers (n = 25) and non-smokers (n = 25), 37 years (range 20–55 years) years; controls: females (n = 10), males (n = 10), non-smokers, 30 years (range 20–50 years) Peripheral blood lymphocytes	• **TL:** exposed (17.46 ± 1.99 µm) vs. unexposed (12.55 ± 0.82 µm); **Sig. (Mean and SD).** • **TI:** exposed (81.49 ± 4.31%) vs. unexposed (76.01 ± 3.70%); **Sig. (Mean and SD).** • **TM:** exposed (14.31 ± 2.16 µm) vs. unexposed (9.78 ± 0.91 µm); **Sig. (Mean and SD).** • Combined: exposed (1.31 ± 0.15) vs control (1 ± 0.07).	[71]
Kopjar et al. *	2009	Antineoplastic drugs	Croatia	–	100 (50 exposed, 50 unexposed) Healthcare workers (females smokers and non-smokers; 37.00 ± 8.87 and 37.98 ± 8.96 years in exposed and controls, respectively). Peripheral blood lymphocytes	• **TL:** exposed (17.46 ± 0.08 µm) vs. unexposed (14.00 ± 0.02); **Sig. (Mean and SEM).**	[70]
Ladeira et al.[1]	2015	Antineoplastic drugs	Portugal	Cyclophosphamide (4.6% samples, LOD =0.10 and LOQ=0.30), 5-Fluorouracil (10.7% samples, LOD =3.30 and LOQ=10) and Paclitaxel (21.7% samples, LOD =0.167 and LOQ=0.50) surface contamination (µg/cm^2)	92 (46 exposed, 46 unexposed), Hospital workers (females and males, smokers and non-smokers, 33.85 ± 1.21 and 39.26 ± 1.42 years in exposed and controls, respectively Peripheral blood lymphocytes	• **TI:** exposed (15 ± 1.40) vs. unexposed (12.41 ± 1.24); **Non-sig. (Mean, type of variability not reported).**	[25]

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Table 1 (*continued*)

Author	Year	Main Chemical Exposure	Country	Exposure Assessment or Biomarkers of Exposure	Population Characteristics	DNA Damage	Reference
Laffon et al.[2]	2005	Antineoplastic drugs (cyclophosphamide, cisplatin, doxorubicin, mitomycin C, 5-fluoro-uracil, methotrexate)	Portugal	–	52 (30 exposed, 22 unexposed), Nurses (females and males, smokers and non-smokers, 33.30 ± 9.20 and 44.09 ± 8.20 years in exposed and controls, respectively Peripheral blood lymphocytes	• **TL:** exposed (46.46 ± 0.09 µm) vs. unexposed (42.68 ± 0.10 µm); **Sig. (Mean and SEM).**	[65]
Maluf et al.	2000	Antineoplastic drugs	Brazil	–	24 (12 exposed, 12 unexposed; historic control of 34 non-exposed workers), Hospital workers (exposed: 34.75 ± 5.42, controls:34.42 ± 4.48; Historical controls: 29.13 ± 7.98 years old) Peripheral blood lymphocytes	• **VS:** exposed (20.83 ± 10.19) vs. unexposed (8.08 ± 5.16), **Sig. (Mean and SD).**	[33]
Ness et al.	2021	Antineoplastic drugs	Brazil	–	64 (29 exposed, 35 unexposed) Pharmacists, pharmacy technicians and nurses (females and males, smokers and non-smokers, 41.00 (37.0–48.0) and 42.00 (31.00 – 54.00) years in exposed and controls, respectively). Peripheral blood leukocytes	• **TI:** exposed (10.42 ± 0.18) vs. unexposed (10.35 ± 0.25); **Non-sig. (Mean and SEM).**	[29]
Oltulu et al.	2018	Antineoplastic drugs (cyclophosphamide, doxorubicin, 5-fluorouracil, paclitaxel)	Turkey	–	59 (29 exposed, 30 unexposed) Health workers (females and males, smokers and non-smokers, 32.28 ± 7.41 and 34.67 ± 8.65 years in exposed and controls, respectively). Peripheral blood lymphocytes	• **VS:** exposed [2.00; (0.00–3.00) vs. unexposed [0.00; (0.00–2.25)]; **Non-sig. (Median and 25th–75th quartiles).**	[66]
Sasaki et al.	2008	Antineoplastic drugs	Japan	–	224 [121 exposed, 57 highly exposed (antineoplastic preparation), 46 unexposed], Female nurses (exposed: 37 ± 10, controls:36 ± 9) Peripheral blood lymphocytes	• **TL (log units):** exposed (0.764 ± 0.121) vs. unexposed (0.711 ± 0.089); **Sig.** • **TM (log units):** exposed (0.312 ± 0.253) vs. unexposed (0.253 ± 0.237); **Non-sig.**	[72]
Rekhadevi et al.	2007	Antineoplastic drugs	India	Urinary cyclophosphamide (42 out of 60 workers had detectable levels in urine) Mean 0.44 ± 0.26 µg/mL (range 0.08 – 0.9 µg/mL)	120 (60 exposed and 60 unexposed) Nurses (females, non-smokers, 38 ± 6 years) Peripheral blood leukocytes	• **TL:** exposed (13.66 ± 2.37) vs. unexposed (6.21 ± 0.0.92); **Sig. (Mean and SD).**	[64]
Rombaldi et al.	2009	Antineoplastic drugs	Brazil	-	40 (20 exposed and 20 unexposed) Hospital workers (females and males, smokers and non-smokers, 31.50 ± 9.34 and 28.23 ± 6.30 years in exposed and controls, respectively). Peripheral blood leukocytes	• **VS:** exposed (18.89 ± 8.62) vs. unexposed (6.21 ± 2.78); **Sig. (Mean and SD).**	[28]
Ündeğer et al.[3]	1999	Antineoplastic drugs	Turkey	–	60 (30 exposed and 30 unexposed) Nurses (29 females and 1 male in each group, smokers and non-smokers, 29 ± 5 (range 20–42) and 29 ± 5 (range 19–43) years in exposed and controls, respectively). Peripheral blood lymphocytes	• **VS:** Undamaged: 137.6 ± 42.9 vs. Slightly damaged: 36.7 ± 22.0 vs Damaged: 16.2 ± 19.5 vs Highly damaged: 9.4 ± 9.2 Controls - Undamaged: 176.0 ± 19.6; Slightly damaged:15.2 ± 13.0; Damaged: 4.1 ± 5.9; Highly damaged: 5.7 ± 6.1; **Sig. (Mean and SD)**	[67]
Ursini et al.	2006	Antineoplastic drugs	Italy	5-Fluorouracil (5-FU), cytarabine (CYA),	65 (35 exposed, 30 unexposed),	• **TM:** pharmacy technicians (20.8 ± 10.1 µm) vs.	[69]

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Table 1 (*continued*)

Author	Year	Main Chemical Exposure	Country	Exposure Assessment or Biomarkers of Exposure	Population Characteristics	DNA Damage	Reference
				gemcitabine (GCA), cyclophosphamide (CPA) and ifosfamide (IFO) surface contamination (μg) Pharmacy: 5-FU= 10.5, CPA= 9.5, IFO= 20, GCA and CYA= 0) Day hospital unit: 5-FU= 85, CPA= 10, IFO= 14, GCA and CYA= 0) Total antineoplastic surface contamination value (expressed as the sum of all 5 measured drugs and referred to the sum of all examined surfaces) was 4 μg/m^2 in the pharmacy and 18 μg/m^2 in the day hospital unit. Biological monitoring of α-fuoro-β-alanine in urine (metabolite of 5-fuorouracile) LoD= 0.018 μg/mL 30 and 1.140 μg/L in two-day hospital nurses and 20 μg/ in a ward nurse.	Hospital workers [males, females, smokers and non-smokers, Pharmacy technicians (n = 5): 35.8 ± 9.9; Day hospital nurses (n = 12): 37.6 ± 5.5; Ward nurses (n = 13): 32.7 ± 7.7; Controls:34.9 ± 8.5 years old) Peripheral blood lymphocytes	hospital nurses (15.5 ± 9.0 μm) vs. Ward nurses (14.7 ± 7.9 μm) vs. unexposed (16.1 ± 8.1μm); **Non-sig. (Mean and SD).**	
Yoshida et al.	2006	Antineoplastic drugs (cyclophosphamide, dacarbazine, isophosphamide, aclarubicin, amrubicin, bleomycin, daunorubicin, doxorubicin, pirarubicin, carboplatin, cisplatin, docetaxel, etoposide, irinotecan, paclitaxel, vinblastine, vincristine, vinorelbine, rituximab)	Japan	umu assay from surface contamination Different antineoplastics mixtures handled/sampling day, with genotoxicity ratio to blank from 0.97 to 3.93.	37 (19 exposed, 18 unexposed), Female nurses (smokers and non-smokers, 29.2 (range 21–48) and 31.6 (range 21–47) years in exposed and controls, respectively) Peripheral blood lymphocytes	• **TL:** exposed (8.5, ranging 4.5–13.6 μm) vs. unexposed (5.1, ranging 3.5–10.3 μm); **Sig. (Mean and range).**	[73]
Villarini et al.	2011	Antineoplastic drugs	Italy	5-Fluorouracil and cytarabine surface contamination: [n = 22 wipes (29.3%) were positive for 5-FU or CYT (LoD 0.01 μg/mL, for both chemicals), with concentrations ranging from 0.02 to 2 μg /dm^2. The highest contamination level was found in the preparation labs, with 36% positive wipe samples (concentration range: 0.13–2 μg /dm^2), with respect to the administration wards, with 21.2% positive samples (concentration range: 0.02–0.48 μg /dm^2)]. Urinary cyclophosphamide: [levels in the post-shift urine samples of 7 nurses, with CP concentrations in the range 0.1–0.2 μg/mL; n = 1 had a urinary CP concentration of 1.2 μg/L. The remaining samples had CP below the limit of detection.	104 (52 exposed, 52 unexposed) Healthcare workers (females and males, smokers and non-smokers, 39.26 ± 9.59 and 36.21 ± 11.21 years in exposed and controls, respectively) Peripheral blood leukocytes	• **TL:** exposed (2.73 ± 0.28) vs. unexposed (1.67 ± 0.14); **Sig. (mean and SEM).**	[19]

SD – Standard Deviation; SEM – Standard Error of Mean; TI – Tail Intensity (% DNA in tail); TL – Tail Length; TM – Tail Moment; VS – Visual Scoring.
* Updated studies from the same author/group of authors. In the first paper, the authors report the mean and SD as 17.46 ± 1.99 and 12.55 ± 0.82 for the exposed and controls, respectively. However, these data are at odds with the calculated SEM in the 2009 paper (i.e. 0.08 and 0.02 in exposed and controls, respectively). Based on the reported group size the SEM should be 0.28 (exposed, n = 50) and 0.18 (controls, n = 20), respectively.
[1]Results are obtained from graphs (variation assumed to be SD).
[2]Results are reported in tables, but the variation is surprisingly low and inconsistent in the publication. For the meta-analysis we have used results as follows: exposed (46.46 ± 2.65) vs controls (42.68 ± 1.89). See the supplement for further discussion of the results.
[3]The results are reported as raw data in four comet classes. For the meta-analysis, we have calculated the total visual score as: Nundamaged*0 + Nslightly damages*1 + Ndamaged*2 + Nhighly damaged*3.

manipulation and handling of antineoplastic drugs; however, it is also important to address in future studies 'forgotten' workers who also handle these drugs, such as housekeeping personnel, laundry workers, workers who deal with transportation and storage, as well the families of patients undergoing therapy [74].

Human biomonitoring studies encompass environmental/exposure monitoring, biomarkers of exposure, biomarkers of effect and individual/susceptibility biomarkers [2,3,74]. Few studies gathered in this systematic review used exposure assessment – just one study with urinary biomarkers and three a combination of exposure assessment and biological monitoring. The majority relied on control groups, without known exposure to antineoplastic agents. The use of biomarkers of internal exposure would lead to more robust conclusions, since it would be possible with greater certainty to associate the biomarkers of effect with a specific exposure [75,76]. For that purpose, according to the type of exposure, half-life of the compounds and work dynamics (shift duration and rotations), the best time points for sample collections should be studied [77].

The studies that used environmental monitoring showed inconsistent results. From the three studies that used environmental monitoring and biomarkers of internal exposure, only the study from Villarini et al. [19] showed statistically significant differences between exposed and controls. The studies from Connor et al. [59] and Ursini et al. [69] showed no differences, despite an increase of DNA damage in the exposed group in the latter. Rekhadevi et al. [64] showed significant differences in DNA damage between exposed and controls and used a urinary internal exposure biomarker. Regarding surface contamination exposure assessment, Yoshida et al. [73] showed significant differences in DNA damage between groups, while Ladeira et al. [25] did not, but found a slight increase in DNA damage in exposed persons. Overall, results from the studies that only have a reference group showed statistically significant differences between exposed and controls.

Nevertheless, it is clear that there is a relationship between the effect size and high risk of comet assay measurement bias/exposure misclassification and region of the study, since middle-income countries have shown higher effect size than studies from high-income countries. The higher effect sizes reported in studies conducted in middle-income countries may partly reflect differences in the implementation and adherence to occupational safety standards and limited enforcement capacity. In these settings, the use of personal protective equipment might be inconsistent, procedures for the safe handling, preparation, and disposal of antineoplastic agents might be inadequately followed, and access to appropriate engineering controls, such as biological safety cabinets, might be limited. Additionally, Good Laboratory Practices and

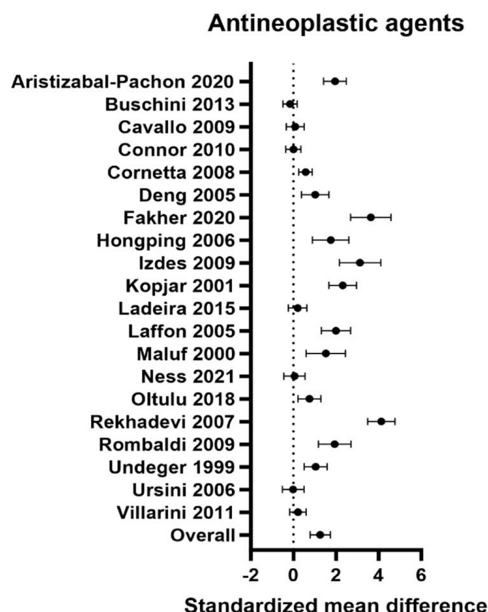

Fig. 2. Forest plot of studies that assessed the difference between DNA strand breaks (according to comet assay test) in all healthcare workers handling antineoplastic drugs in comparison with controls. The forest plot has been reproduced for graphical purposes. Original Forest and Funnel plots are included in Supplementary Figs. S1 and S2, respectively.

Fig. 3. Traffic light plot of risk of bias or exposure misclassification, being D1 – Comet assay description; D2 – Assay control; D3 – Blinded scoring; D4 – Exposure misclassification.

Table 2
Risk of bias or exposure misclassification versus middle/high-income countries.

Item	Low risk (green)	Moderate risk (yellow)	High risk (red)	Statistics
Comet assay description (D1)	6/7	0/2	3/2	Low vs moderate/high: $\chi^2 = 0.02$ (P = 0.89) Low/moderate vs high: $\chi^2 = 0.61$ (P = 0.44)
Assay control (D2)	0/1	None	9/10	Not applicable
Blinded scoring (D3)	5/2	None	4/9	Low vs high: $\chi^2 = 3.04$ (P = 0.08)
Exposure misclassification (D4)	3/0	1/0	5/11	Not applicable
Overall	1/0	3/1	5/10	Low/moderate vs high: $\chi^2 = 3.3$ (P = 0.07)

Table 3
Sensitivity analysis of effect sizes in studies according to use of blinded analysis, inclusion of assay controls and description of comet assay procedure. Results are mean and 95% confidence intervals, and the number of studies.

Item (risk of bias)	Antineoplastic drugs studies (Mean, 95% CI) (n)
Blinding	
High	1.26 (0.69, 1.83) (n = 13)
Low	1.19 (0.31, 2.06) (n = 7)
Inclusion of assay controls	
High	1.15 (0.67, 1.61) (n = 19)
Low	1.94 (1.72, 2.70) (n = 1)
Comet assay description	
High[a]	1.79 (0.31, 3.28) (n = 5)
Moderate[a]	2.76 (1.10, 4.42) (n = 2)
Low	0.73 (0.34, 1.13) (n = 13)
Expo sure assessment	
High	1.47 (0.89, 2.06) (n = 16)
Moderate[b]	0.29 (−0.12, 0.70) (n = 1)
Low[b]	0.08 (−0.16, 0.31) (n = 3)
Combined	
High	1.42 (0.78, 2.06) (n = 15)
Moderate[c]	0.58 (−0.05, 1.20) (n = 4)
Low[c]	0.21 (−0.17, 0.60) (n = 1)

[a] High and moderate risk groups have been combined due to low number of observations (2.07, 95% CI: 0.82, 3.31). [b] Moderate and low risk groups have been combined due to low number of observations (0.13, 95% CI: −0.08, 0.33). [c] Moderate and low risk groups have been combined due to low number of observations (0.48, 95% CI: 0.01, 0.96).

formal safety guidelines may exist but that does not necessarily ensure consistent and effective implementation; they are often adapted from high-income settings but applied in contexts with limited financial, technical, specialized human resources, and less systematic continuous training of healthcare workers [78–80]. Another aspect to be considered is the possibility of high exposure levels and workplace characteristics, such as lower levels of automation, longer shifts, greater time pressure in healthcare settings that may increase the likelihood of accidental exposure.

To our knowledge, the first meta-analysis concerning the induction of DNA damage in professionals occupationally exposed to antineoplastic agents was published in Sakhvidi et al. [46]. The authors included 14 studies in the overall meta-analysis, which produced an effect size of 1.93 (95% CI: 1.15, 2.71). However, the authors considered the analysis to be without a clear conclusion because of heterogeneity in the subjects' characteristics, comet assay protocols, and exposure levels. Another meta-analysis with the same objective [27], showed a significant association between occupational exposure to antineoplastic agents and DNA damage. Nineteen papers were included in the quantitative evaluation, and the effect size (ES) was calculated based on the mean, standard deviation (SD), and sample size provided for each study. The pooled effect size was 1.27 (95% CI: 0.66–1.88), very similar to that found in the current study. However, after a quality assessment of the studies, 14 studies (published in 13 papers) were included in a separate analysis and the effect size decreased to 0.92 (95% CI: 0.26–1.59). The effect size in studies where professionals used no or limited protective equipment was higher than in studies where personal and environmental protective equipment (such as an airflow cabinet) was used. In the same direction as we observed in the current meta-analysis, the effect size in studies conducted in Asia was higher than in those performed in America and Europe, with the lowest effect size observed in European studies. The authors suggested that this can be explained by differences in procedures, protocols, education, and legislation across the world. The effect size was similar to that in the pooled analysis when considering work task (other health professionals different from nurses were considered as controls). The authors stated that the value of heterogeneity remained stably high even though several sub-group analyses were performed.

The three systematic review and meta-analyses have reported relatively different results in terms of effect size: 1.93 (95% CI: 1.15, 2.71; n = 14), 1.27 (95% CI: 0.66, 1.88; n = 19) and 1.18 (95% CI: 0.72, 1.65; n = 20), in Sakhvidi et al. [46], Gianfredi et al. [27], and our review, respectively. At first sight, it may not come as a surprise because new papers have been incorporated in updated meta-analyses. However, there are more substantial differences that relate to inclusion of studies and the selection/use of relevant results. The overlap of studies in the Sakhvidi et al. [46] and Gianfredi et al. [27] meta-analyses is 78% (14 out of 18 studies). With regard to our review, there is 61% (7 out of 18 studies) and 80% (16 out of 20 studies) overlap between the included studies in our meta-analysis and those by Sakhvidi et al. [46] and Gianfredi et al. [27], respectively. The discrepancy between Gianfredi et al. [27] and our meta-analysis is the publications by Kopjar et al., 2009 [70] (excluded because of an overlap in results with Kopjar et al., 2001 [71]), Sasaki et al. [72] (excluded due to lack of information on variability), Yoshida et al. [73] (excluded due to log-transformed results) and Oltulu et al. [66] (i.e., included in our review but not in the meta-analysis by Gianfredi et al. [27]).

In addition to differences in inclusion of studies, there are also differences in the results used for the meta-analysis. Below, we have assessed reasons for the discrepancy in reported effect sizes in the three meta-analyses. We wish to emphasize that pointing fingers at other authors is not the purpose, but discrepancies originate from differences in the interpretation of reported results and errors. For both causes, it is interesting and relevant to assess the consequences to gain better understanding of factors of uncertainty in meta-analyses. Supplementary Table S1 shows the reported SMDs in the three meta-analyses. The studies are ordered according to the variability in results (lowest to highest variability assessed as sum of squares). These results were used in the meta-analysis by Sakhvidi et al. [46] and Gianfredi et al. [27]. However, Villarini et al. [19] also report results from three other comet descriptors in a figure, and the central tendency and variability values are not readily available (results where the DNA migration is based on the mean of individual comets, and tail length as either mean or median values of individual comets). Therefore, abstracting only the reported mean and SD, and taking into account that it was only one out of four ways to convey information about the level of DNA strand breaks, biases the result toward increased effect size in the meta-analysis. The other studies have given rise to more variability in effect sizes, which appears to have been due to other reasons as described below.

Sasaki et al. [72]. The publication is not included in our meta-analysis because the results are reported as log-transformed data.

Table 4
Assessment of courses of variability in meta-analyses on DNA strand breaks by exposure to antineoplastic agents in human biomonitoring studies.

Author	Year	SMD (Sakhvidi 2016)	SMD (Gianfredi, 2020)	SMD (This study)	Variation (SS)[a]	Observations
Cornetta	2008	0.57	0.57	0.57	0.00	Simple data (%TDNA)
Connor	2010	−0.01	−0.01	0.00	0.00	Null effect finding (pooled comet descriptors)
Rekhadevi	2007	4.14	4.14	4.12	0.00	Simple data (tail length)
Ladeira	2015	NR	0.31	0.29	0.00	Simple data (%TDNA)
Rombaldi	2009	NR	1.98	1.94	0.00	Simple data (visual score)
Izdes	2009	3.19	3.19	3.13	0.00	Simple data (visual score)
Maluf	2000	1.58	1.58	1.52	0.00	Simple data (visual score)
Ursini	2006	−0.01	0.11	−0.01	0.01	Null effect finding (pooled exposure groups)
Yoshida	2006	1.39	1.56	NR	0.01	Difference in variation in the control group
Cavallo	2009	NR	−0.18	0.08	0.03	Null effect finding (pooled comet descriptors)
Sasaki	2008	0.15	0.47	NR	0.05	Use of log-data or back-transformed results
Villarini	2011	0.66	0.66	0.21	0.10	Pooled comet descriptors
Hongping	2006	NR	2.50	1.75	0.28	Pooled comet descriptors
Kopjar	2001	1.32	1.32	2.31	0.49	Pooled comet descriptors
Buschini	2013	−0.16	−1.33	−0.16	0.68	Gianfredi has used the reported SEM as SD
Deng	2005	NR	2.91	1.02	1.80	Pooled comet descriptors
Undeger	1999	1.05	−1.71	1.04	3.80	Transformation of original results in four classes of comets (visual scoring system) to a central value
Kopjar	2009	7.35	2.45	NR	12.0	Difference in variation (SEM rather than SD)
Laffon	2005	7.91	7.95	1.58	22.5	Difference in variation (SEM rather than SD)

[a] The variation is reported as sum of squares [SS = Sum $(X_i - \text{mean})^2$] to give an impression of the magnitude of difference in effect size between studies. The SS values correspond to differences between two values (present meta-analysis compared to previous analyses, or Sakhvidi vs Gianfredi). Not reported (NR).

However, Gianfredi et al. [27] and Sakhvidi et al. [46] report different SMDs, which is due to direct use of log-transformed data or results that have been back-transformed to mean and SD on ordinary scale.

Yoshida et al. [73]. The discrepancy between SMDs in Gianfredi et al. [27] and Sakhvidi et al. [46] appears to be due to a difference in SD of the control group (1.70 vs 1.07, respectively). At first sight, the similarity in numbers suggests a typographical error. However, we are not able to verify this because Yoshida et al. [73] does not report SD and none of the reported data in the publication have these values (i.e., mean, median and range).

Buschini et al. [18]. The discrepancy is entirely due to transformation of the reported SEM value to SD (i.e., present study and Sakhvidi et al. [46]) as compared to using the SEM in the meta-analysis (i.e. Gianfredi et al. [27]).

Undeger et al. [67]. The results used in meta-analyses by Sakhvidi et al. [46] and Gianfredi et al. [27] are not identical to any results in the original publication. We do not know where the results come from as the authors have not clarified the path from the original results to those reported in the meta-analysis. The reason for this might be that the original results were reported as number of comets in different classes of the visual scoring system (in this case four classes). We have used the standard procedure to calculate the overall visual score in arbitrary units (exposed: 298.6 ± 76.5; controls 237.3 ± 30.7 arbitrary units, mean ± SD). This produces a SMD (1.04), which is actually similar to that reported by Sakhvidi et al. [46] (1.05), even though the results are quite different (exposed: 0.49 ± 0.38; control: 0.19 ± 0.15). The meta-analysis by Gianfredi et al. [27] has other primary results (exposed: 105.5 ± 36; controls 153.8 ± 18.3), which produces an inverse effect (SMD = −1.33). It appears that the transformation of primary comet assay results has given rise to heterogeneity in calculations of central values of DNA migration.

Laffon et al. [65]. The discrepancy between meta-analyses rests entirely on an interpretation of the variation in the results. Previous meta-analyses have used the tabulated SD as measure of variability. However, this variability is unexpectedly low (i.e. it gives rise to a coefficient of 1% in the control group). In addition, the variability is larger for comet results in figures in the same publication. It indicates that the variability is reported as SD, although it is actually SEM. This interpretation is supported by assessment of Funnel plots where the study looks like an outlier if the variability is used as SD instead of SEM (see supplement figures). Therefore, we have used the results as SEM (Supplementary Table S2).

Kopjar at al. [70]. The reason for the discrepancy between meta-analysis is uncertain, although it appears to be rooted in use of SD and SEM values in only the exposed group. In the control group, the original results are reported as 14.00 ± 0.02 (mean and SEM), which is correctly converted to 14.00 ± 0.14 (mean and SD, n = 50). However, the transformation of results in the exposure group from the original report (17.46 ± 0.08, mean and SEM, n = 50) has produced different results in Sakhvivi et al. [46] (17.46 ± 0.65, mean and SD) and Gianfredi et al. [27] (17.46 ± 1.99, mean and SD). Moreover, both of these transformations from the original SEM values are too high (0.08 * SQRT (50) = 0.57). It should be noted that the original publication report in three different places the SEM to be 0.08 in the exposure group.

Combined, the assessment implies that there are different sources of variability in meta-analyses in the present dataset(s). Uncomplicated results typically give rise to the same SMD. Pooling of results from different exposure groups or comet descriptors is a factor that may give rise to some heterogeneity between meta-analyses. However, inconsistency in the use of SEM and SD appears to be a factor that gives rise to the largest heterogeneity in effect sizes.

The present review as well as previous reviews have certain uncertainties, which should be acknowledged.

i) Comet assay results describe the amount of DNA migration from the nucleus to the tail of the comets. As descriptors depend on the assay condition, staining and image analyses, they do not directly correspond to DNA damage levels. Moreover, several comet descriptors are commonly used. There is no experimental evidence to exclude data on tail length, tail intensity, tail moment or visual score from meta-analyses. Unfortunately, the descriptors do not give rise to the same SMD. The example is the results by Hongping et al. [60] (Supplementary Table S3), which give rise to SMDs of 2.39 (95% CI: 1.42, 3.36) and 0.73 (95% CI: −0.01, 1.48) for tail length and tail moment, respectively. The results originate from the same set of comets, so they cannot both be accurate indicators of the difference between the exposed subjects and controls. It is a conservative approach to assume that the value, which most accurately correlates to the difference in DNA

damage, is between the extremes (in the present example as the mean of the two results, SMD = 1.52).

ii) The assessment of risk of bias shows severe deficits in the reporting of positive assay controls (19 out of 20 studies) and blinded scoring (13 out of 20 studies). Lack of reporting of these does not mean necessarily that the studies did not include assay controls and/or coded samples/slides before analysis. However, it is difficult to understand why authors would choose to omit important information that increases the quality of the study. In theory, reporting of positive assay controls is probably associated with lower effect size because there is a stronger reason to demonstrate the reliability of the comet assay if the results are not statistically significant. In a systematic review of heavy metal exposures, we have observed higher effect size for studies with high risk of bias related to assay controls [47], whereas there was no difference between studies with low and high of bias for studies on pesticide exposure [48]. For blinding/coding of samples, bias is expected to occur when investigators know the exposure status of the samples. However, our previous systematic reviews and meta-analyses have not demonstrated a consistent relationship as studies on heavy metals showed low risk of bias was associated with high effect size [47], whereas the opposite was observed for studies on pesticide exposure [48]. It is very important that papers include information on blinding/scoring of samples and report results on assay controls.

iii) The meta-analysis is based on standardized results. It is a valid statistical analysis on dissimilar endpoints, but SMD may not be interpreted intuitively as it refers to the difference in effect between groups as standard deviation units. Effect sizes can also be reported more understandably as ratios, but this approach has the limitation that the comet descriptors must be positive values. Studies with no DNA damage must be excluded as would be the case for Oltulu et al. [66] in the present review because the median of the control group is zero.

5. Conclusion

The current meta-analysis shows that exposure to antineoplastic drugs is associated with increased levels of DNA strand breaks in human leukocytes. The meta-analysis confirms the overall results by Sakhvidi et al. [46] and Gianfredi et al. [27] using the same statistical analysis, with a different procedure for obtaining central tendencies and variability of the included studies. Analysis of overall effect sizes indicates a relatively large heterogeneity, which can be ascribed to several factors, foremost inconsistency in the abstracted variability (i.e. SD or SEM). The overall SMD in our meta-analysis is relatively close to the SMD obtained by non-parametric analysis (1.18 and 1.03, respectively), indicating come consistency in the statistical analysis. Subgroup and sensitivity analysis tend to be statistically underpowered, although there is evidence to suggest that effect sizes are higher in middle-income countries as well as studies with high risk of comet assay measurement bias and exposure misclassification.

In summary, DNA damage measured by comet assay is linked to recent or ongoing exposures, as the detected DNA damage can be repaired. This particular characteristic can be regarded as an advantage in occupational biomonitoring since the workers are continuously exposed due to their professional activity. For future, it is important to align with minimum information requirements, especially for reporting HBM [81,82] to assure accurate interpretation and comparability of biomarkers of exposure and effect.

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mrrev.2026.108590.

Data availability

Data will be made available on request.

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