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GENOTOXICITY AND CARCINOGENICITY

Buccal micronucleus cytome assay to evaluate cyto-genotoxic effects of occupational exposure to antineoplastic drugs: application on a large sample size of workers furnished by an Italian network of oncological hospitals

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Abstract

Many antineoplastic drugs (ADs) used to treat cancer are characterized by the non-selective effect representing a possible cause of health effects in exposed workers. We established an Italian Network of seven Oncological Hospitals with the aim to evaluate, on a large size sample of workers, cyto-genotoxic effects by a sensitive and non-invasive biomarker also detecting workplace and personal contamination. We performed Buccal Micronucleus Cytome (BMCyt) assay on 200 workers handling ADs and 150 controls. AD contamination was detected performing workplace and personal monitoring of Gemcitabine, Ifosfamide, Cyclofosfamide and 5-Fluorouracil, using UHPLC MS/MS. We found in all the exposed group higher mean values of cells with micronucleus (MN‰), higher percentage of positivity to MN (subjects with micronucleated cells frequency exceeding a fixed cut-off value (1.5‰)), higher frequency of binucleated cells, broken eggs and total anomalies than in the controls. Taking into account the tasks (preparation, administration in Day Hospital and wards, administration in room operator and disposal), only preparators and administrators showed higher MN‰ frequency than in controls, whereas each task group showed a similar higher percentage of MN positives than in controls. We found low levels, but still detectable, of contamination in all the monitored workplaces. This study demonstrated induction of genotoxicity and of cytokinesis defect/arrest in buccal cells of workers handling antineoplastic drugs. The BMCyt assay was demonstrated to be a suitable biomarker of effect for biomonitoring of workers handling AD due to its high sensitivity and non-invasivity.

Keywords Antineoplastic drugs · Buccal cells · Micronucleus · Genotoxicity · Cytotoxicity

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Introduction

The EU strategic framework on health and safety at work 2021–2027 (COM(2021) 323) highlighted the need to protect healthcare workers exposed to hazardous medical products (HMPs). HMPs are defined as medicinal products that contain one or more substances that meet the criteria for classification as: Carcinogenic (category 1A or 1B), Mutagenic (category 1A or 1B) or Toxic for reproduction (category 1A or 1B). Several antineoplastic drugs (ADs) are HMP together with some antivirals, hormones, hormonal antagonists, immunosuppressants, antibiotics and other therapeutic groups.

Many AD used to treat cancer bind to DNA or damage it, other drugs interfere with cell growth, proliferation or synthesis of DNA with a nonselective effect, therefore, such drugs could represent a possible cause of health effects in workers who prepare, administer and dispose them. A lot of studies are available on the evaluation of genotoxic effects of ADs on workers handling these drugs using different biomarkers of effect (Huang et al 2023; Vanneste et al 2023). Huang et al 2023 performed a meta-analysis of cytogenetic biomarkers to evaluate the association between exposure to ADs and cytogenetic damage to healthcare workers. They found a significant association between occupational exposure to ADs and cytogenetic damage reviewing studies published between 2005 and 2021 that used cytogenetic biomarkers in health care workers handling ADs. Also Vanneste et al 2023 reviewed the literature relative to the genotoxicity of occupational exposure to ADs. They found in 62 studies (selected starting from 245 retrieved papers) that this kind of exposure can lead to genotoxic damage. Most of the used biomarkers are represented by the comet assay, Chromosomal Aberrations, Sister Chromatid Exchange and MN assay on lymphocytes or buccal cells as biological matrix to detect cytogenotoxic effects of AD. Chromosomal Aberration resulted in the most sensitive and reliable assay (Huang et al 2023 and Vanneste et al 2023), however, it needs more time and more sophisticated and expensive instrumentation with software ad hoc, therefore, it is more difficult to use it in biomonitoring campaigns in comparison with other assays. The Micronuclei (MN) assay was performed mainly on lymphocytes and only lower studies were performed on buccal cells (Burgaz et al. 1999, Cavallo et al 2005; Rekhadevi et al. 2007; Rodríguez-Montero et al. 2016; Ursini et al. 2019; Santos et al. 2020). The buccal micronucleus cytome (BMcyt) assay is able to detect genetic damage, cell proliferation, differentiation and death in exfoliated buccal cells particularly attractive because buccal cells can be collected in a minimally invasive manner (Bolognesi et al 2013). This assay has been demonstrated to be a promising biomarker of effect particularly interesting to evaluate genotoxic effects of potential exposure to antineoplastic drugs (Ursini et al. 2019). One of the limitation of the few available studies on buccal cells is the size of the studied population most of these studies were performed on workers whose number was below 50, except for the study published by Rekadhevi et al. 2007 (on 60 exposed workers). In addition, another limitation of the available studies is that only very few of them evaluated also surface and personal AD contamination (Cavallo et al 2005 and Ursini et al. 2019), some studies such as Rekadhevi et al. (2007) and Santos et al. (2020) didn't perform environmental monitoring, but they measured only Cyclophosphamide in the urine.

Several studies demonstrated AD workplace contamination, despite the availability of guidelines and the use of closed-system device for the preparation of such drugs, causing potential exposure of workers handling them particularly during the preparation and administration tasks (Palamini et al 2020). For example, a recent Italian survey monitoring program performed in nine Italian Hospital centers performed over five years (2016–2021), found detectable levels of ADs with a decreasing trend (Sottani et al 2022).

We established an Italian Network of seven Oncological Hospitals to be able to perform a multicenter study whose aim was the evaluation of the potential early cyto-genotoxic effects of occupational exposure to a mixture of antineoplastic drugs on a large sample size using buccal cells as a no-invasive biological matrix. We also aimed to evaluate the occupational exposure occurring in the hospitals involved in the biomonitoring study detecting, three weeks before the buccal cell collection, workplace and personal contamination due to Gemcitabine, Ifosfamide, Cyclofosfamide and 5-Fluorouracil using all the same experimental procedure. Final aim was to contribute to the validation process of the Buccal Micronucleus Cytome (BMCyt) assay.

Methods

Subjects

We enrolled workers handling mixtures of ADs and controls from an established Italian Network of seven Italian hospitals. We included, among the potentially exposed subjects, workers involved in the AD administration, preparation, cleaning and workers of operating rooms by performing the Hyperthermic intraperitoneal chemotherapy (HIPEC) procedure and pressurized intraperitoneal aerosol chemotherapy (PIPAC). We performed data collection by a questionnaire including information on age, gender, smoking habits and job seniority after that all persons gave their informed consent prior to their inclusion in the study. The study was

approved by the Ethical Committees of the involved hospitals and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Buccal Micronucleus Cytome (BMCyt) assay

The exfoliated buccal cells, obtained by scraping the right and left cheeks with a wet toothbrush (previously immersed in Phosphate Buffer Solution), after washing out the mouth with water, were collected at the start-shift of Wednesday. The cells were suspended in 25 ml of buffer solution containing 0.01 M Tris − HCl, 0.1 M EDTA and 0.02 M NaCl (pH 7), and sent to the laboratory that performed the BMCyt assay where they arrived within 24 h. The cells were washed twice in the buffer solution. Then 50 µl of the final cell suspension (1.5×10^6–2×10^6 /ml) were dropped on pre-warmed slides (37 °C). Cells air dried were fixed in 80% methanol for 48 h. Orange acridine (0.005%, Sigma) was used as staining and the analysis was undertaken by fluorescence microscope at 400 X magnification (Leica, Germany). At least 2000 differentiated cells were analysed for each subject by two expert readers according to the criterion established by Titenko-Holland et al. (1998). We recorded the presence of cells with MN, nuclear buds (NB) and broken eggs (BE), all indicative of DNA damage, binucleated cells (indicative of cytokinesis defect or arrest), karyolytic cells (in advanced stage of necrosis and apoptosis) and cells with condensed chromatin (in early stages of apoptosis). For each subject, the frequency of each abnormality was estimated on total differentiated exfoliated cells and expressed as ‰. Moreover, subjects with micronucleated cell frequency exceeding a fixed cut-off value (1.5‰) were considered positive to the MN assay. We chose a 1.5 MN‰ threshold on the basis of the results of HUMNXL (Human MicroNucleus project on eXfoLiated buccal cells) published by Bonassi et al. (2011) that show the estimated spontaneous MN frequency of 0.74‰ (95% CI 0.52–1.05). HUMNXL project involved 5424 subjects obtained from 30 laboratories worldwide that used different staining methods. In particular, for our staining method (Acridine Orange) a mean value of 0.98‰ (95% CI 0.39–1.14) was reported, therefore, we established a cut-off value of 1.5 above both the upper limits of confidence intervals.

Workplace and personal monitoring

AD contamination was detected performing workplace and personal monitoring of four drugs (Gemcitabine, Ifosfamide, Cyclofosfamide and 5-Fluorouracil) analysed by ultra-high-performance liquid chromatographic method coupled with a tandem mass spectrometer (UHPLC MS/MS), according to the method described by Sottani et al 2022 and Pt compounds were analysed by ICP-MS by the Environmental Research Center, Istituti Clinici Scientifici Maugeri IRCCS, of Pavia. The LOD of Gemcitabine, Ifosfamide and Cyclofosfamide is 0.1 ng, whereas 5-FU and Pt compounds LOD are 5 ng and 0.008, respectively.

Biological monitoring

Ultra-high-performance liquid chromatographic method coupled with a tandem mass spectrometer (UHPLC MS/MS) was used to analyse α-fluoro-β alanine, Gemcitabine, Cyclofosfamide and Ifosfamide, whereas Pt compounds were analysed by ICP-MS by the Environmental Research Center, Istituti Clinici Scientifici Maugeri IRCCS, of Pavia. The Lod of α-fluoro-β alanine is 1.0 ng/ml, the LOD of Gemcitabine was 0.2 ng/ml, Ifosfamide and Cyclofosfamide LOD was 0.1 ng/ml, whereas Pt compounds LOD is 0.01 ng/ml.

Statistical methods

Statistical analysis was performed using IBM SPSS software version 25. The chi-square test and Fisher's exact test were used to assess the significance of associations between categorical variables and the groups analyzed. Non-parametric tests (Mann–Whitney U test and Kruskal–Wallis test) were employed to assess the significance of differences in mean values between the exposed and control subgroups. Pairwise comparisons were conducted using Dunn's procedure with a Bonferroni correction for multiple comparisons. Multiple regression analysis was performed, using the studied biomarkers of effect as dependent variables, and exposure and confounders, such as age, gender, and smoking habits, as independent variables. A p-value of < 0.05 was considered statistically significant.

A sensitivity analysis was also carried out to assess whether the effect of exposure on ‰ cells with MN varied across different hospitals. This was done by sequentially excluding one hospital at a time and re-running the regression models.

A logistic regression model was also applied to evaluate the effect of exposure to antiblastic drugs, adjusting for age, gender, and smoking habits. In this analysis, the outcome variable was the result of the micronucleus test, classified as either positive or negative. The model allowed us to estimate the odds ratios (OR) and their corresponding 95% confidence intervals (95% CI) for each independent variable, enabling the identification of any significant risk factors for a positive micronucleus test. The Pearson correlation coefficient was also used to evaluate the possible correlation of ‰ Cells with MN with age in the whole sample and in both the control group and the exposed group.

Results

In Table 1, we show the general characteristics of the studied population that resulted to be prevalently female (77.4% in the exposed group and 71.3% in controls). The deeper analysis, performed according to the specific task, showed a lower percentage of females (57.4%) in the group of preparators ($n = 54$) in respect to the other tasks. As shown in Table 1, the studied population with smoking habits was 19.2% in the exposed group and 12.2% in the group of controls with no statistically significant differences. Regarding the age of the studied groups, we did not find any difference between exposed and controls, whereas the group of operating room was younger (32.67 years) than the other groups, with a statistically significant difference.

Cyto-genotoxicity by BMCyt Assay

Table 2 shows the results of the BMCyt assay with the mean values of all the analysed parameters referred to all the exposed subjects, with respect to the control group and to the other groups, obtained taking into account the task (preparators, administrators, workers of the operating room and disposer/cleaners). The table shows that the genotoxic parameters and the total anomalies mean values resulted all higher in the exposed group compared to controls. In particular, relatively to the frequency of cells with MN (‰ Cells with MN), analysing the results according to the specific task, it resulted higher in the groups of preparators and administrators compared to the control group. The comparison among the tasks didn't find differences relatively to cells with more than one MN, or cells with NB + BE, whereas MN + BE + NB mean frequency values resulted higher not only in preparators and administrators, but also in workers of operating rooms. Table 2 also shows the mean frequency values of karyolitic and binucleated cells and of cells with condensed chromatin. Among these cytotoxicity parameters, we found statistically significant differences only for binucleated cells mean value, which was higher in the exposed group compared to controls. The frequencies of cells with Condensed Chromatin were resulted higher in the exposed group, but without statistical significance. Whereas total anomalies mean values were higher in the preparators and administrators with respect to controls.

The same table also presents the BMCyt assay results for each hospital that participated in the biomonitoring. It shows the mean frequency values of all BMCyt assay parameters in controls vs exposed subjects, indicating that almost all hospitals (with the exception of one) had higher mean values of ‰ cells with MN in the exposed group and, in four of them, the differences were statistically significant.

Table 3 shows the results obtained by the multiple regression model considered performed to estimate the effect of antineoplastic drugs exposure on biomarkers outcomes also considering confounders, such as age, gender and smoking habits. It showed a significant effect of *exposure* to antineoplastic drugs on ‰ Cells with MN, ‰ Cells with more than one MN, ‰ Tot Anomalies and ‰(MN + NB + BE) (Table 3). Indeed, exposed workers had a, respectively: 0.66%, 0.19%, 1.76% and 0.99% increase in the above mentioned biomarkers, compared to non-exposed workers, with a statistically significant effect ($p < 0.05$). An increase of 0.47% in ‰ cells with Condensed Chromatin among males is also registered ($p < 0.001$), as well a significant effect of age on ‰(MN + NB + BE) ($p = 0.039$).

In addition, relatively to the biomarker ‰ Cells with MN, we also analyzed its correlation with age, and we didn't find it neither in the whole sample (Pearson = 0.034; *P*-value = 0.530) or in both the control group (Pearson = − 0.003; *P*-value = 0.976) and exposed group (Pearson = 0.077; *P*-value = 0.281).

We conducted an additional analysis, presented in Table 4, which summarizes the regression model results after removing certain covariates. This sensitivity analysis concerns to the ‰ Cells with the MN biomarker outcome. The effect of antineoplastic drugs exposure on ‰ cells with MN remains significant across all models, confirming a robust association. The findings show that the coefficient B for exposure ranged from 0.537 to 0.762, depending on which hospital was excluded. Specifically, excluding Hospital A resulted in the lowest B value (0.537), while excluding Hospital E led to the highest (0.762). In all other cases, B remained between 0.568 and 0.725, indicating some variability but consistently maintaining statistical significance ($p < 0.001$).

Regarding the MN positivity, we found that the percentages of MN positive subjects was higher in the exposed group compared to controls (21.5 vs 4.7) and also the analysis performed taking into account the specific task confirmed the differences between each exposed group (20.4%, 22.0%, 20.0% and 25% in preparators, administrators, operating room and disposer/cleaners respectively) and controls.

Table 5 shows the results of the logistic regression model used to evaluate the effect of exposure to antineoplastic drugs adjusted for age, gender and smoking habits considering the positivity vs. negativity to MN as an outcome variable. We found that workers exposed to antineoplastic drugs are 6.4 times more likely to have positivity to MN compared to unexposed, adjusting for age, gender, and smoking habits. The effect is statistically significant ($p < 0.001$). In addition, being male are less likely to have micronucleus induction than females; this effect is statistically significant ($p = 0.042$). There are non-significant effects due to age and smoking habits.

Table 1 Characteristics of the studied populations and statistical analysis of the differences

Variables		Total $N=350$	Controls $N=150$	Exposed $N=200$	Preparators $N=54$	Administrators $N=123$	Operating room $N=15$	Disposal $N=8$	*P*-Value	
									Exposed vs. Controls	Task comparison
		N (%)	*N* (%)	*N* (%)	*N* (%)	*N* (%)	*N* (%)	*N* (%)		
Gender	Males	88 (25.2)	43 (28.7)	45 (22.6)	23 (42.6)	17 (13.9)	4 (26.7)	1 (12.5)	0.197[a]	0.001[a]
	Females	261 (74.8)	107 (71.3)	154 (77.4)	31 (57.4)	105 (86.1)	11 (73.3)	7 (87.5)		
Smoking habit	Yes	52 (16.2)	17 (12.2)	35 (19.2)	4 (8.0)	26 (23.2)	3 (23.1)	2 (28.6)	0.228[a]	0.133[b]
	No	240 (74.8)	108 (77.7)	132 (72.5)	39 (78.0)	79 (70.5)	9 (69.2)	5 (71.4)		
	Former	29 (9.0)	14 (10.1)	15 (8.2)	7 (14.0)	7 (6.3)	1 (7.7)	0 (0.0)		
Age	Mean ± SD	42.36 ±10.73	43.27 ±10.37	41.70 ±10.97	43.41 ±11.65	41.63 ± 10.42	32.67 ±7.46	48.13±11.98	0.171[c]	0.002[d]*
	[range]	[22–66]	[23–65]	[22–66]	[25–66]	[22–66]	[25–50]	[30–63]		
Job seniority	Mean (years ±SD)	9.15 ±8.27	7.30±7.45	9.36 ±8.35	8.84 ±7.96	10.36±8.73	3.01±1.51	8.75±7.80	0.301[c]	0.057[d]
	[range]	[0.08–32]	[0.33–30]	[0.08–32]	[0.08–28]	[0.25–32]	[1–6]	[1–22]		

[a]$\chi 2$ test

[b]Fisher's exact test

[c]Mann–Whitney Test

[d]Kruskal–Wallis Test

*Dunn's procedure with a Bonferroni correction for multiple comparisons. Operating room vs Controls ($p=0.002$), vs Preparators ($p=0.007$); vs. Administrators ($p=0.019$); vs. Disposal ($p=0.010$)

Table 2 Buccal Micronucleus Cytome Assay results: genotoxic and cytotoxic effects

	Genotoxity (Mean value ± SD)				Cytotoxicity (Mean value ± SD)			
	‰MN	‰Cells with more than one MN	‰(NB + BE)	‰(MN + NB + BE)	‰Karyolitic cells	‰Binucleated cells	‰ Cells with Condensed Chromatin	‰Total Anomalies
Controls (N = 150)	0.36 ± 0.63	0.05 ± 0.21	1.38 ± 2.07	1.73 ± 2.19	106.45 ± 62.47	3.93 ± 2.45	0.45 ± 0.89	6.16 ± 4.02
Exposed (N = 200)	0.98 ± 1.41	0.25 ± 0.72	1.67 ± 1.92	2.64 ± 2.35	102.42 ± 67.88	4.32 ± 2.21	0.59 ± 1.08	7.78 ± 3.84
P-value	**< 0.001[c]**	**0.002[c]**	**0.017[c]**	**< 0.001[c]**	0.284[c]	**0.032[c]**	0.147[c]	**< 0.001[c]**
Preparators (N = 54)	1.14 ± 1.75	0.37 ± 0.95	1.91 ± 2.34	3.05 ± 2.61	100.33 ± 70.01	4.64 ± 2.26	0.50 ± 0.73	8.55 ± 3.84
Administrators (N = 123)	0.92 ± 1.26	0.21 ± 0.62	1.46 ± 1.67	2.39 ± 2.19	103.24 ± 69.85	4.20 ± 2.19	0.66 ± 1.24	7.43 ± 3.83
Operating room (N = 15)	0.91 ± 1.29	0.06 ± 0.15	2.28 ± 1.89	3.18 ± 1.98	91.74 ± 36.29	4.78 ± 2.24	0.24 ± 0.57	8.25 ± 3.87
Disposal (N = 8)	0.86 ± 1.35	0.42 ± 0.82	2.01 ± 2.29	2.87 ± 3.29	124.00 ± 73.32	3.18 ± 1.79	0.65 ± 0.95	7.12 ± 3.77
P-value	**< 0.001[d]**	**0.012[d]**	**0.029[d]**	**< 0.001[d]**	0.670[d]	**0.041[d]**	0.367[d]	**< 0.001[d]**
	*Contr vs. Prep/Adm			*Contr vs. Prep/Adm/ Operating room				*Contr vs. Prep/Adm
Hospital A								
Controls (N = 20)	0.58 ± 1.11	0.09 ± 0.24	1.61 ± 1.94	2.19 ± 2.12	77.73 ± 40.88	4.73 ± 3.01	0,60 ± 0.92	7.61 ± 3.96
Exposed (N = 34)	1.68 ± 1.89	0.63 ± 1.05	0.76 ± 0.84	2.45 ± 2.03	92.58 ± 70.89	4.06 ± 2.02	0.68 ± 0.86	7.82 ± 3.73
P-value	**0.035[c]**	0.066[c]	0.136[c]	0.518[c]	0.338[c]	0.554[c]	0.585[c]	0.610[c]
Hospital B								
Controls (N = 3)	0.45 ± 0.45	0.00 ± 0.00	0.46 ± 0.45	0.91 ± 0.78	65.19 ± 20.98	3.82 ± 0.86	0.00 ± 0.00	4.74 ± 1.29
Exposed (N = 18)	0.86 ± 1.57	0.24 ± 0.70	0.50 ± 0.83	1.37 ± 1.69	67.74 ± 33.79	3,29 ± 1.71	0.29 ± 0.57	5.18 ± 2.18
P-value	0.814[c]	0.669[c]	0.534[c]	0.887[c]	0.961[c]	0.605[e]	0.471[c]	0.743[e]
Hospital C								
Controls (N = 26)	0.17 ± 0.36	0.00 ± 0.00	1.07 ± 0.98	1.25 ± 1.01	122.45 ± 74.11	3.69 ± 1.57	0.16 ± 0.50	5.10 ± 2.09
Exposed (N = 27)	0.39 ± 0.45	0.01 ± 0.06	2.39 ± 2.89	2.78 ± 3.02	129.18 ± 76.41	4.35 ± 2.59	0.23 ± 0.50	7.38 ± 4.38
P-value	**0.037[c]**	0.326[c]	**0.021[c]**	**0.009[c]**	0.695[c]	0.803[c]	0.515[c]	**0.039[c]**
Hospital D								
Controls (N = 34)	0.27 ± 0.48	0.00 ± 0.00	1.19 ± 2.52	1.47 ± 2.55	104.07 ± 60.47	3.12 ± 2.91	0.38 ± 0.70	4.97 ± 4.95
Exposed (N = 31)	1.28 ± 1.00	0.09 ± 0.23	2.61 ± 1.80	3.89 ± 2.36	94.47 ± 83.45	3.60 ± 2.36	0.72 ± 0.91	8.18 ± 3.27
P-value	**< 0.001[c]**	**0.016[c]**	**< 0.001[c]**	**< 0.001[c]**	**0.034[c]**	0.180[c]	0.133[c]	**< 0.001[c]**
Hospital E								
Controls (N = 18)	0.53 ± 0.70	0.20 ± 0.30	0.93 ± 1.33	1.46 ± 1.64	138.48 ± 73.46	4.35 ± 2.08	0.74 ± 1.56	6.76 ± 3.21
Exposed (N = 21)	0.32 ± 0.48	0.05 ± 0.15	1.24 ± 1.25	1.56 ± 1.44	143.02 ± 77.94	4.30 ± 2.15	1.82 ± 2.27	7.73 ± 3.75
P-value	0.512[c]	0.174[c]	0.321[c]	0.626[c]	1.000[c]	0.939[e]	**0.024[c]**	0.477[c]

Table 2 (continued)

Hospital F								
Controls (N = 35)	0.31 ± 0.54	0.06 ± 0.31	2.25 ± 2.72	2.56 ± 2.93	109.53 ± 62.82	4.71 ± 2.22	0.49 ± 0.89	7.83 ± 4.52
Exposed (N = 50)	0.90 ± 1.36	0.21 ± 0.64	2.01 ± 2.08	2.91 ± 2.43	99.93 ± 52.13	4.90 ± 2.12	0.25 ± 0.52	8,27 ± 4.37
P-value	**0.022**[c]	0.110[c]	0.865[c]	0.183[c]	0.796[c]	0.692[e]	0.220[c]	0.384[c]
Hospital G								
Controls (N = 14)	0.48 ± 0.52	0.03 ± 0.12	0.62 ± 0.61	1.11 ± 0.74	83.53 ± 28.08	2.70 ± 2.13	0.52 ± 0.67	4.37 ± 1.92
Exposed (N = 19)	1.09 ± 1.90	0.49 ± 1.19	1.39 ± 1.38	2.48 ± 1.98	89.50 ± 42.12	5.35 ± 1.79	0.54 ± 0.73	8.86 ± 3.33
P-value	0.843[c]	0.077[c]	0.152[c]	**0.011**[e]	0.649[e]	**0.001**[e]	1.000[c]	**< 0.001**[e]

The bold values indicates stastically significant values

c Mann–Whitney Test

d Kruskal Wallis Test

e Student's *t*-Test

*Dunn's procedure with a Bonferroni correction for multiple comparisons

Table 3 Multiple regression model estimating the effect of antineoplastic drug exposure on biomarker outcomes, adjusting for confounders

Biomarker	Independent variables				
	Total sample (*N* = 350)				
		Unstandardised B	95% CI	Standardised Beta	*P-Value*
‰Cells with MN	Age	0.008	–0.004 – 0.020	0.068	0.212
	Gender[a]	–0.163	–0.451 – 0.125	–0.061	0.266
	Smoking habits[b]	–0.117	–0.287 – 0.053	–0.074	0.177
	Exposure[c]	0.662	0.402 – 0.921	0.273	**< 0.001**
‰Cell with more than one MN	Age	0.001	–0.005 – 0.007	0.023	0.679
	Gender	–0.083	–0.224 – 0.059	–0.064	0.251
	Smoking habits	0.020	–0.064 – 0.103	0.026	0.640
	Exposure	0.190	0.063 – 0.318	0.164	**0.004**
‰Total Anomalies	Age	0.039	–0.003 – 0.081	0.100	0.071
	Gender	0.020	–0.987 – 1.027	0.002	0.969
	Smoking habits	–0.286	–0.880 – 0.309	–0.052	0.345
	Exposure	1.764	0.855 – 2.673	0.212	**< 0.001**
‰(MN + NB + BE)	**Age**	0.026	0.001 – 0.050	0.114	**0.039**
	Gender	–0.086	–0.671 – 0.500	–0.016	0.773
	Smoking habits	–0.199	–0.545 – 0.147	–0.063	0.258
	Exposure	0.989	0.461 – 1.518	0.204	**< 0.001**
‰ Condensed Chromatin	Age	–0.002	–0.013 – 0.008	–0.026	0.640
	Gender	0.475	0.226 – 0.724	0.207	**< 0.001**
	Smoking habits	0.048	–0.100 – 0.195	0.035	0.525
	Exposure	0.201	–0.024 – 0.426	0.098	0.079

[a] Baseline: Female; [b] Baseline: non-smoker; [c] Baseline: unexposed

Table 4 Sensitivity analysis: Multiple regression model results on ‰Cells with MN biomarker

Model	Exposure[c] (B, 95% CI)	*p*-value
Baseline Model (all covariates)	0.662 (0.402; 0.921)	< 0.001
Without Smoking habits	0.627 (0.382; 0.872)	< 0.001
Without Age	0.640 (0.383; 0.898)	< 0.001
Without Gender	0.672 (0.414; 0.930)	< 0.001
Without Hospital A	0.537 (0.292; 0.781)	< 0.001
Without Hospital B	0.677 (0.415; 0.939)	< 0.001
Without Hospital C	0.725 (0.428; 1.021)	< 0.001
Without Hospital D	0.568 (0.258; 0.878)	< 0.001
Without Hospital E	0.762 (0.473; 1.051)	< 0.001
Without Hospital F	0.686 (0.386; 0.986)	< 0.001
Without Hospital G	0.662 (0.398; 0.925)	< 0.001

[c] Baseline: unexposed

Workplace contamination

A total of 789 wipes (249 in the pharmacy units, 524 in the local of administration and 16 in the operating rooms of a Unit that performed HIPEC/PIPAC) were collected.

A total of 195 workers wore pad tests on left and right forearms and thorax ($n = 585$ pads).

Figure 1 shows the percentages of wipes resulted containing detectable values of the detected drugs. Among all the collected wipes (789) we found that Gemcitabine and Pt compounds resulted the drugs present in the highest percentage (58% and about 96% respectively). The second and third panel show the percentages of contaminated wipes in the pharmacy units where the preparation occurs and in the locals of administration. Gemcitabine was present in 48.6% of the wipes collected in the pharmacy units and in a higher percentage (64.7%) in those used in the local administration. Whereas Ifosfamide was present in a higher percentage in the pharmacy units (35%) compared to that found in the local administration (25%). The other detected drugs were distributed with similar percentages in the two kinds of workplaces. The last panel of Fig. 1 shows the percentages of wipes with detectable values of ADs of the operating room where we found higher percentages of contaminated wipes for 5-FU and Pt compounds, in particular, Pt compounds were found in 100% of the sampled wipes.

We found, on collected wipes low, but still detectable levels of contamination in all the monitored workplaces with a slightly higher percentage of wipes positive (higher than lod) to Gemcitabine in the administration locals. In the operation rooms we found Pt compounds in 100% of the collected wipes although in very lower concentrations in respect to the other drugs.

However, Pt compounds were present in almost all the detected pads and it could also be explained by the very lower lod (0.008 ng) in respect to the higher lod of the other drugs.

Figure 2 shows personal monitoring results. We found that 5-FU and Pt compounds were detectable in the highest percentage. In particular, the figure shows higher percentages with at least one positive pad for GEM, IFO, CP and 5-FU for preparators, whereas for Pt compounds, the percentage of positive workers was higher among administrators.

Detection of ADs in urine

We analysed the urine of about 50% of the enrolled exposed workers and they all resulted with α-fluoro-β alanine, Gemcitabine, Cyclofosfamide, Ifosfamide and Pt compounds values below the limit of detection.

Discussion

This study represents one of the few available ones that performed Buccal Micronucleus Cytome (BMCyt) assay to evaluate cyto-genotoxic effects of AD potential exposure

Table 5 Logistic regression model predicting the likelihood of positivity to MN outcome based on gender, age, smoking habits and exposure

Outcome: Positivity to MN					
Independent variables	Coefficient (β)	Odds Ratio (OR)	95% CI		*p*-value
			Lower	Upper	
Intercept	− 3.579	0.028			< 0.001
Female		Ref			
Male	− 0.965	0.381	0.150	0.965	0.042
Non-smoker		Ref			
Former smoker	− 0.800	0.449	0.098	2.060	0.303
Smoker	− 0.789	0.454	0.164	1.255	0.128
Age	0.019	1.019	0.988	1.051	0.232
Unexposed		Ref			
Exposed	1.864	6.452	2.610	15.950	< 0.001

on health care workers simultaneously with the environmental and personal monitoring of drug contamination. We demonstrated genotoxic effects in terms of induction of all genotoxic parameters in agreement with the presence of workplace and personal contamination with Gemcitabine, Ifosfamide, Cyclofosfamide, 5-Fluorouracil and Pt compounds found in all the detected area.

Most of the available studies demonstrated genotoxic effects evaluated on lymphocytes by different biomarkers of effects such as Chromosomal Aberration, Comet Assay, Sister Chromatid Exchange Assay and Micronucleus assay (Huang et al 2023; Vanneste et al 2023).

The present study confirms on a large size sample the results obtained previously by our laboratory (Cavallo et al 2005 and Ursini et al. 2019) on a lower number of subjects. In fact, our first study was performed on 30 exposed workers (five pharmacy technicians and 25 nurses administrating ADs) and 30 controls working in one Oncological Hospital using MN assay on lymphocytes and buccal cells. The study had demonstrated induction of MN only on buccal cells particularly in workers involved in the administration of ADs in addition, in all the exposed workers we also found Chromosomal Aberrations induction. Our second study (Ursini et al. 2019) enlarged the sample size (45 exposed and 45 controls) involving three Oncological poles and increased the kind of the detected anomalies since we performed Buccal Micronucleus Cytome assay that allows to detect both cytotoxic and genotoxic damage. The study found in preparators and administrators, a higher frequency of cells with MN, in addition, it found an increase of NB, indicative of gene amplification, in preparators and an increase of cells with Condensed Chromatin, indicative of apoptosis, in the administrators. Moreover, in the preparators and administrators similar higher percentages of MN positive subjects were found.

With the constitution of a network of seven hospitals, we have had the possibility to enroll a higher number of potentially exposed workers reaching 200 subjects to compare with 150 controls increasing, therefore, the statistical power. In the present study, we confirmed the induction of MN in exposed workers in terms of the mean value of cells with MN in comparison with controls. Also the results of the detection of cyto-genotoxic effects analysed in each hospital showed a higher frequency of cells with MN in the exposed group compared to controls in most of the participating hospitals (six out of seven) although the statistically significance was found in four out of seven hospitals. In particular, the lack of statistically significant difference between exposed and controls was found in the hospitals with the lowest number of subjects, confirming

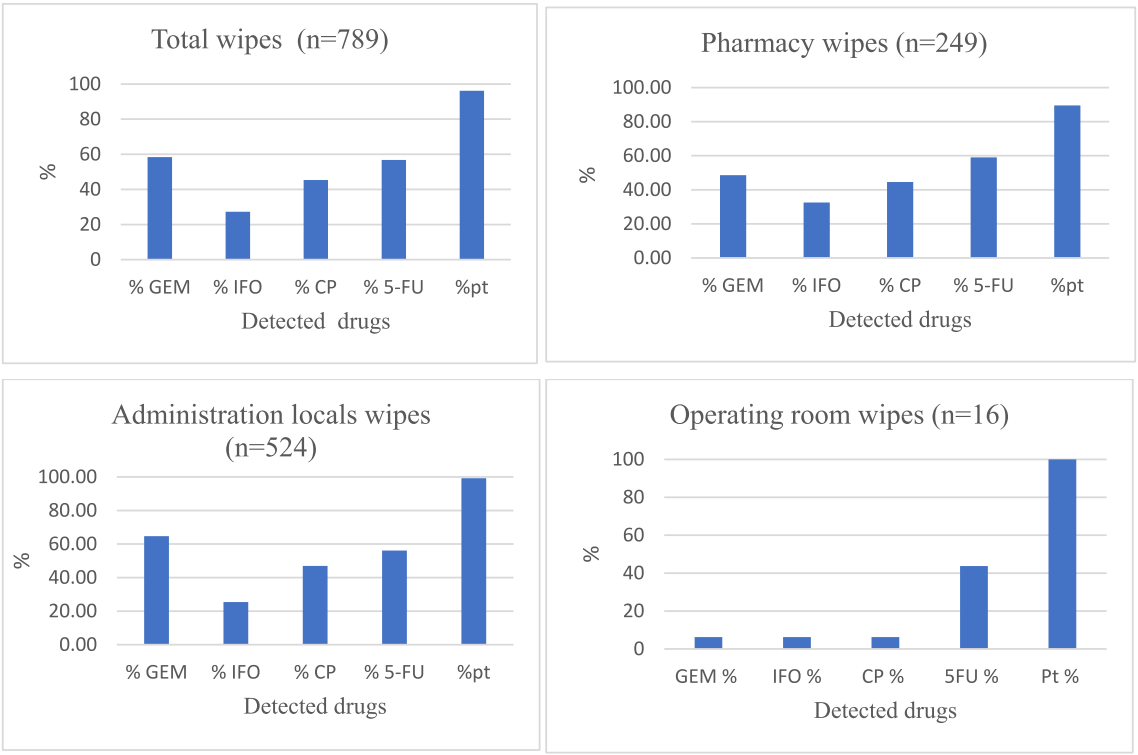

Fig. 1 Percentage of wipes with detectable drug concentration calculated for all the collected wipes (first panel) and for each detected workplace (the other panels)

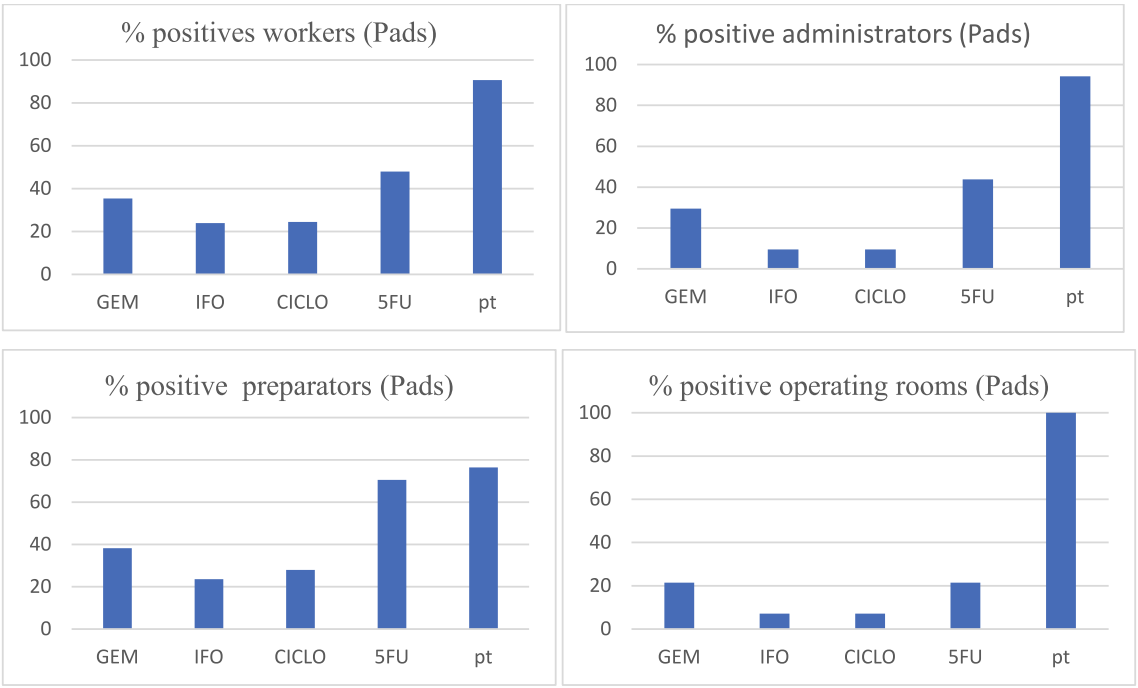

Fig. 2 Percentages of all monitored workers with at least one positive pad (first panel) and percentages only of administrators, preparators and operating rooms with at least one positive pad (other panels)

the need to perform biomonitoring studies in large-size samples. In addition, the sensitivity analysis concerning ‰ cells with MN confirmed a robust association of this biomarker with the exposure.

When we took into account the specific tasks, we found higher mean values of all the genotoxic parameters in all the tasks (administrators, preparators, workers of operating rooms and disposers/cleaners), but the differences were statistically significant compared to controls only for preparators and administrators. However, the analysis performed in terms of MN positivity showed that in all the exposed groups there were similar and higher percentages of MN positives in respect to the control group. We confirmed the results obtained in Ursini et al. 2019), where for the first time we used an arbitrary cut-off of 1.5‰, chosen on the basis of the results obtained by Bonassi et al 2011. In fact, also in this study we found similar and higher percentages of MN positives in preparators and administrators with respect to the control group, although with higher values. The MN positivity demonstrated to be useful also in other of our previous studies evaluating the genotoxic effects of occupational exposure to nanomaterial such as SiO_2 and graphene-based materials (Ursini et al 2021 and Cavallo et al 2022).

Regarding Broken eggs and Nuclear buds (NB + BE) indicative of genic amplification we found an induction in the group of all the exposed workers, but we did not find statistically significant differences among the different groups of exposed subjects according the specific task, (probably due to the low number of subjects) with higher (NB + BE) values in the two groups operating room and disposal (dispenser/cleaners). Taking into account all the genotoxic parameters (MN + NB + BE), we found not only differences between exposed and controls, but analysing the tasks, we found significant differences for administrators, preparators and workers of operating rooms with higher mean values in respect to the control group. In Ursini et al. 2019) we have found induction of cells with Condensed Chromatin in exposed workers whereas in the present study the increase of cells with Condensed Chromatin was in the exposed group not statistically significant. In the present study, we also found, unlike the previous study, a statistically significant difference in Binucleated cells between exposed and controls demonstrating a cytokinesis defect/arrest in the exposed group without statistically significant differences in the specific tasks.

Our results on MN frequency are in line with those of Rekadevi et al. 2007 who evaluated genotoxic effects of AD exposure using comet assay and Micronucleus assay on both lymphocytes and Buccal cells. They found differences between exposed and controls in all the used biomarkers, however, they analysed only MN frequency differently from our present study which also detected other nuclear and cellular anomalies. Also Santos et al. 2020 found an increase of MN frequency on buccal cells of both pharmacy and nursery workers in Brazil. A Cuban study performed by Rodríguez-Montero et al. 2016 found in a small group of exposed

workers ($n = 14$) higher mean value of micronucleated cells in respect to those found on the same number of unexposed subjects but the difference was not statistically significant, however, the mean value of micronucleated cells found in Chemotherapy Ambulatory's Room workers ($n = 10$) was higher than that found on workers involved in the preparation of cytostatic drugs ($n = 4$), although the difference was only near the statistical significance.

Relatively to other studies evaluating drugs contamination in the operating rooms where workers performed hyperthermic intraperitoneal chemotherapy (HIPEC) procedure and a pressurized intraperitoneal aerosol chemotherapy (PIPAC), Ndaw et al 2018 detected Platinum drug from various locations in operating rooms to evaluate the potential exposure of the medical staff during a HIPEC and a PIPAC procedure. They also found significant workplace contamination, but in more than 50% of urine samples platinum was below the limit of quantification (< 10 ng/L). Also Roussin et al 2021 found Cisplatin and doxorubicin contamination on the operating room surfaces even after a cleaning protocol. Another study assessed and compared knowledge and practices about the safe handling of ADs by workers handling ADs in operating rooms performing HIPEC/PIPAC and workers of compounding units. They found AD contamination in the operating room and the Authors conclusions of the study highlight the need to improve training programs for all the workers handling ADs including those in operating rooms (Delafoy et al 2023).

To the best of our knowledge, our study represents the first one evaluating genotoxic effects on buccal cells of AD administration in operating rooms, although with the limitation relative to the low number of workers in respect to the workers performing other tasks, so it is necessary to enlarge sample size to confirm our results relative to the higher mean value of MN + NB + BE and percentage of MN positive subjects in comparison with the control group.

The multiple regression model considered to estimate the effect of antineoplastic drugs exposure on biomarkers outcomes also considering confounders, such as age, gender and smoking habits, demonstrated that for most of the genotoxicity parameters only the exposure influenced their mean values except for ‰(MN + NB + BE) that was influenced also by age and Condensed Chromatin that was influenced only by the gender. In addition, the Pearson correlation did not find any influence of age on the frequency of cells with MN neither in the whole sample, nor in the exposed group or in the control group. The lack of influence of age on MN frequency found in the present study is also slightly in contrast with those of Bonassi et al 2011 because the mean age of our population was 42.3 ± 10.7 years, near the lower value of the age class (40–49 years) that resulted increased in the Bonassi et al. 2011 study.

In addition, Bonassi et al 2011 demonstrated that smoke influenced MN frequency only in heavy smokers (more than 40 cigarettes/day) whereas among the smoker subjects involved in our present study there were no smokers of more than 40 cigarettes/day.

Relatively to the influence of confounding factors on the positivity to MN, the results of the logistic regression model to evaluate the effect of exposure to ADs adjusted for age, gender and smoking habits on the outcome variable "positivity vs. negativity to MN", showed an influence of sex with females more susceptible than males to the MN positivity, whereas we found a lack of effects of age and smoking habits on MN positivity.

We confirmed the presence of detectable levels of all the examined ADs as found by other recent studies such as the one performed in Canada by Palamini et al 2020, the study performed in France (Ndaw et al. 2023) and the study performed in nine Italian hospitals by Sottani et al 2022. We found surfaces contaminated, particularly by Pt compounds, also in the operating rooms confirming the results of other studies such as Ndaw et al 2018 that, however, found the presence of Pt in urine (limit of quantification was 10 ng/L) in less than 50% of the exposed subjects performing HIPEC and PIPAC. In the urine of workers in operating rooms, we did not find detectable levels of ADs differently by Ndaw et al 2018, probably because of the low exposure levels.

In conclusion, the present study furnishes not only data on early genotoxic effects of AD occupational exposure evaluated by the no-invasive Buccal Micronucleus Cytome assay, but also assessed simultaneously the exposure by wipes and pads differently from the most used way to evaluate exposure that consists mainly with the administration of a questionnaire. The advantage of this study is the large population size compared with previous studies, including ours, and it demonstrates that BMCyt assay is a good biomarker to evaluate early genotoxic effects of exposure to antineoplastic drugs since the evaluated parameters are correlated with workplace and personal exposure measurements so, it could furnish a useful contribution to the BMCyt assay validation process.

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Author contribution Cinzia Lucia Ursini: Conceptualization, Methodology, Formal analysis, Writing—original draft preparation, Writing—review and editing; Supervision; Emanuela Omodeo-Salè: Methodology, Resources, Investigation, Funding acquisition, Supervision; Giorgia Di Gennaro: Investigation; Giuliana Buresti: Formal analysis, Writing—original draft preparation, Writing—review and editing; Anna Maria Fresegna: Investigation; Aureliano Ciervo: investigation; Marco Gentile: investigation; Raffaele Maiello: investigation; Sabrina Beltramini: Resources, Investigation, Supervision; Daniela Gaggero: Investigation; Nicoletta Rigamonti: Resources, Investigation, Supervision; Erica Maccari: Investigation; Giorgia Zorzetto: Investigation;

Piera Maiolino: Resources, Investigation, Supervision; Pasquale Di Filippo: Investigation; Maria Concetta Bilancio: Investigation; Paolo Baldo: Resources, Investigation, Supervision; Valeria Martinello: Investigation; Andrea Di Mattia: Resources, Investigation, Supervision; Chiara Esposito: Investigation; Patrizia Nardulli: Resources, Investigation, Supervision; Maria Rita Laforgia: Investigation; Delia Cavallo: Conceptualization, Methodology, Writing—original draft preparation, Writing—review and editing; Supervision.

Data availability All relevant data are included in the manuscript. Further inquiries may be directed to the corresponding author.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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