Current Genetic Medicine Reports (2018) 6:144–154
https://doi.org/10.1007/s40142-018-0149-6

CYTOGENETICS (T LIEHR, SECTION EDITOR)

Micronuclei and What They Can Tell Us in Cytogenetic Diagnostics

Galina Hovhannisyan[1] · Tigran Harutyunyan[1] · Rouben Aroutiounian[1]

Published online: 27 September 2018

Abstract

Purpose of Review The micronucleus (MN) assay is a validated method of genetic toxicology, widely used for human biomonitoring studies. This review summarizes and discusses current data regarding involvement of MN in pathogenesis of different diseases, potential of MN assay to be used as cytogenetic diagnostic technique, as well as highlights current achievements in studies concerning clinically relevant chromosomal instability using MN assay.

Recent Findings Recent studies suggested that MN are indicator of pathological events in affected as well as not affected "target" tissues of an organism. They can be effectively used in risk assessment and to distinguish stage of pathological manifestations in diseases. Molecular-genetic studies revealed that MN are not only the markers of, but at the same time inducers of genomic instability.

Summary The MN assay is an informative cytogenetic tool, alone and in combination with molecular genetic methods. Although it is not always clear if MN are a result or inducer of pathogenic effects, the vast number of clinical studies substantiated that they have high potential for clinical practice, as they are associated with diseases.

Keywords Chromosomal instability · Cytogenetic diagnostics · Micronucleus (MN) assay · Pathogenesis

Introduction

Genomic medicine is based on the knowledge that virtually every medical condition, disease susceptibility, or response to treatment is caused, regulated, or influenced by genes. Genetic testing therefore has the potential to add value across the whole disease spectrum, ranging from single-gene disorders with a Mendelian inheritance pattern to complex multi-factorial diseases [1]. The relationship between genome stability and human health becomes most obvious in diseases typically characterized by progressive deterioration of specific tissues, susceptibility to cancer, chromosomal rearrangements, and hypersensitivity to genotoxic agents [2, 3]. The identification of disease-causing genetic changes, including chromosomal instability (CIN) is an important diagnostic criterion that contributes to a better understanding of disease etiologies and the choice of treatment [4]. Genetic changes may appear in the early stages of a disease, long before the clinical manifestation, and can serve as prognostic biomarkers. Disease prediction and diagnosis based on genetic testing is a broad field with diverse applications, ranging from karyotyping to screen for gross chromosomal abnormalities, to molecular-genetic-based detection of single nucleotide exchanges. Nowadays implementation of genetic findings in medical practice is not only a highly anticipated approach but also current goal of human genetics studies [5]. Schrodi et al. [5] noted that at present, cancer research, population screening for Mendelian diseases, and pharmacogenetics have benefited the most from the application of genomics. However, genetic-based individual prediction of disease remains very difficult, as many issues have not been resolved yet.

The development of genetic biomarkers for diseases, confirmation of their sensitivity and specificity are being actively realized [4, 6, 7]. At present, new biomarkers are mainly developed based on genomics, epigenetics, transcriptomics, proteomics, and metabolomics [6]. Remarkably, a number of diseases are accompanied by an increased level of chromosomal damage. A universally accepted marker for detection and quantification of genomic instability is the appearance and frequency of

This article is part of the Topical Collection on *Cytogenetics*

✉ Rouben Aroutiounian
rouben_a@hotmail.com

[1] Department of Genetics and Cytology, Yerevan State University, P.O. Box 0025, Yerevan, Armenia

micronuclei (MN); the latter evolves within proliferating cells after chromosome breakage or malsegregation. The MN test is one of the most commonly used cytogenetic methods [8–10]. MN can only be observed in cells completing nuclear division, which can be recognized by their binucleated appearance after cytokinesis blocking with cytochalasin B. This so-called "cytokinesis-block micronucleus assay" (CBMN) has evolved into a more comprehensive method for quantifying chromosomal instability, mitotic dysfunction, and cell death, and denominated as the "cytokinesis-block micronucleus cytome assay" (CBMNCyt) [11].

Quantification of MN frequency is easily performed in each tissue undergoing proliferation, like stimulated lymphocytes or oral mucosa epithelia [8, 9] and epithelial cells of other origin [12, 13]. CBMNCyt in peripheral blood lymphocytes is the most frequently applied biomonitoring method in humans to evaluate genetic instability associated with cancer risk and age-related degenerative diseases [11, 14–16]. The "micronucleus cytome assay in buccal exfoliated cells" (BMNCyt) provides complementary data regarding chromosomal damage and cytotoxic effects in an easily accessible tissue not requiring cell cultivation [11, 17, 18]. It can be used for accelerated aging, cancer, and neurodegenerative disease risk assessment [18, 19].

Elevated levels of MN are indicative of defects in DNA repair and chromosome segregation, which both can result in chromosome instability, e.g., often seen in cancer. Thus, causal relations between an increase of MN frequency and cancer risk are suggested [20, 21] and increased levels of MN can serve as a prognostic or diagnostic biomarker for risk assessment of cancer and other diseases [22–24]. Furthermore, MN are now recognized to be involved in the recently discovered phenomenon chromothripsis [25••, 26••], the latter being suggested to be a potential major contributor to the initiation and development of human cancer.

In this review, we summarize the research on MN frequency in peripheral blood lymphocytes, buccal mucosa, and in other cells, being studied as potential biomarker to identify individuals with certain pathologies and/or with increased risk for different diseases, with main emphasis on papers published since 2015.

Micronucleus Test

MN are small, extranuclear, chromatin-containing bodies surrounded by a nuclear envelope. MN originate from acentric chromosome fragments or whole chromosomes that fail to be included in the daughter nuclei during mitosis [27]. There are four major and two more additional possibilities for the fate of an MN: (i) degradation of the MN or the micronucleated cell, (ii) reincorporation into the main nucleus, (iii) extrusion from the cell, and (iv) persistence in the cytoplasm. Two additional, recently discovered possible fates include (v) premature chromosome condensation/chromothripsis, and (vi) the elimination of micronucleated cells by apoptosis. The available data is still limited, but degradation (i) and extrusion (iii) of MN might occur in rare cases, reincorporation during the next mitosis (ii) occurs more frequently, and the majority of the MN persist without alteration (iv) at least until the next mitosis, possibly much longer. MN exhibit different functional activities, including replication, transcription, and DNA repair [28•].

MN test do not include direct observation of chromosomes; however, the direct correlation between MN formation and genomic damage makes them efficient cytogenetic alternatives to metaphase analysis. The European Centre for the Validation of Alternative Methods reported that the in vitro MN assay is fully validated as an alternative to the chromosomal aberration assay, in a regulatory setting [29]. High concordance between the CBMN and the chromosomal aberration assays was confirmed by analyzing data collected in vitro from 112 structurally diverse potential pharmaceuticals [30].

Originally, MN were evaluated on preparations simply stained by Giemsa. As MN expression requires cell division, CBMN assay was established, and MN were scored in binucleated cells, only. Over time, CBMN assay has evolved into CBMNCyt assay, for simultaneous measuring of DNA damage, cytostasis, and cytotoxicity [8, 27, 31]. CBMNCyt assay is used in cultured human and/or mammalian cells, mainly in lymphocytes, to evaluate DNA damage in only once-divided binucleated cells after induction by a substance to be tested. Evaluation includes (a) number of MN as a marker of chromosome breakage and/or whole chromosome loss; (b) nucleoplasmic bridges (NPBs), as a marker of DNA misrepair and/or telomere end-fusions; and (c) nuclear buds (NBUDs), a marker of elimination of amplified DNA and/or DNA repair complexes. Cytostatic effects are measured via the proportion of mono-, bi-, and multi-nucleated cells, and cytotoxicity via necrotic and/or apoptotic cell ratios [31]. BMNCyt assay is used in non-cultivated human buccal mucosal tissue, to evaluate DNA damage (MN and/or NBUDs), cytokinetic defects (binucleated cells) and proliferative potential (basal cell frequency), and/or cell death (condensed chromatin, karyorrhexis, pyknotic, and karyolytic cells) [9, 17]. The biomarkers measured in CBMNCyt and BMNCyt assays have been associated with increased risks of accelerated aging, cancer, and neurodegenerative diseases [8, 9, 17, 27, 31].

The level of MN expression in lymphocytes and buccal cells depends on various factors, including methodological, demographic, and genetic background, as well as lifestyle and exposure of tested person, which need to be recorded and systematically considered [8]. Inter-individual variability in metabolism or exposures to exogenous and endogenous agents can interfere with MN expression. Methodological

differences in collection of buccal cells, fixation, staining procedures, number of cells counted, and scoring criteria have also proven to affect the results [32]. A study of 200 healthy subjects from Croatian population confirmed an association of the CBMNCyt parameters with age, gender, and lifestyle factors [33]. Such kind of variability in MN results is one of the main limitations in their practical application in clinics [32]; more studies are needed to identify relevant responsible factors, in order to minimize their impact.

The activities of the International Human Micronucleus (HUMN) Project include, among others, evaluation of the association of MN frequency with disease outcome and predicting cancer risk and other diseases in healthy subjects [34, 35].

Micronuclei in Peripheral Blood Lymphocytes, Diagnostic and Prognostic Significance

According to the literature, the CBMN endpoints in peripheral blood lymphocytes (PBL) are sensitive biomarkers being associated with different specific diseases. MN and other nuclear anomalies are biomarkers of genotoxic events and manifestations of CIN often seen in cancer [8, 14, 15, 36, 37]. Data obtained on a large sample of 6718 individuals from ten countries have shown that cancer incidence was significantly higher in tested individuals with medium and high MN frequency [36]. The association between MN frequency and cancer risk in non-hematological malignancies [3, 15] suggests that genome damage events in lymphocytes may also correlate with cancer of other tissues [36]. An increase in the level of MN numbers is also described in autoimmune [38], cardiovascular, metabolic, respiratory, and neurodegenerative disorders [39•].

One of the hallmarks of cancer is CIN, a source of genetic variation in either altered chromosome number or structure. CIN has become a hot topic in recent years, not only for its implications in cancer diagnostics and prognostics, but also for its role in therapeutic response [40]. CBMN endpoints provide a measure of genome damage and/or CIN [41]. The review of Bhatia and Kumar [42] on morphological indicators of CIN in cancers suggests that they may be useful in cancer diagnosis and assessing its behavior.

Studies performed between 2015 and 2018 basically confirm the previously shown sensitivity of CBMN assay in PBL for cancer risk assessment (Table 1). Increase of CBMNCyt endpoints was shown in patients with prolactinoma (pituitary adenoma) [43], bladder cancer [21], and papillary thyroid cancer [44] compared with controls. Based on the level of MN, discrimination may be possible among patients without and with endometrial precancerous lesions and endometrial cancer [45]. CBMNCyt endpoints in breast cancer patients relate with levels of vitamin B6 [46].

CIN is the hallmark of most colorectal cancer (CRC) cases (80–85%). However, clinicians still cannot achieve the best possible disease monitoring, even when a wide panel of markers is used. Data from various studies using PBL samples indicate that MN frequency is a promising biomarker for the early detection and prognosis of CRC. However, more studies are needed in order to describe with certainty the true potential of this biomarker [4]. γH2AX, as marker of DNA DSBs and MN, provides insights into individual genomic instability during progression to CRC, including inflammatory bowel disease as predisposing pathology, and polyps as pre-cancerous state [2]. MN frequency was not predictive for colorectal neoplastic lesions in medium-risk patients; however, nuclear division index (NDI) was significantly lower for CRC patients and may later play a role as a CRC-screening test [20].

Clinical relevance of CBMN parameters confirmed for patients with cervical intraepithelial lesions infected with human papilloma virus (HPV) which is a predisposing factor of malignant transformation [7]. Undiagnosed chronic obstructive pulmonary disease smokers can harbor genetic damage, due to susceptibility to tobacco smoke carcinogens that may be a mediator in lung carcinogenesis [47].

For localization of genomic regions associated with CIN, MN test is applied together with the fluorescence in situ hybridization (FISH) [48•, 49•]. MN and NPB formation were detected in Hodgkin lymphoma cells. FISH painting of chromosomes 9 and 16 demonstrated defects in chromosome segregation and the presence of NPBs. Application of telomere and centromere probes permitted to visualize multiple MN with only centromere sequences (terminal deletion) and MN with telomere and centromere sequences (chromosome lagging). In addition, the presence of telomere and centromere sequences in the NPBs demonstrated the presence of dicentric chromosomes related to telomere fusion. No correlation between chromosomal aberrations and clinical outcomes has been investigated in Hodgkin lymphoma patients. One of the possible mechanisms of genomic instability can be MN formation, that leads to subsequent chromothripsis [48•]. Significantly higher levels of CBMN endpoints were observed in small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) patients compared to controls. Chromosomes 5 and 17 were associated with MN, and chromosomes 5, 18, 20, and 22 were associated with NPBs in SCLC patients. Given the high frequency of chromosomal aberrations observed in SCLCs, chromothripsis can be considered as potential mechanisms for CIN in these patients [49•]. While the studies of Cuceu et al. [48•] and El-Zein et al. [49•] provide data about involvement of chromosomes in spontaneous MN formation in cells of untreated cancer patients, studies on inclusion of chromosomes in MN induced by anticancer drugs could also shed light on possible cytogenetic targets for anticancer therapy [61, 62].

A literature review on presence of chromosome damage in the most common non-communicable diseases (such as

Table 1 CBMNCyt endpoints in lymphocytes of patients reported in publications of 2015–2018

References	Diseases	CBMNCyt endpoints associated with the disease	Clinically relevant markers associated with CBMNCyt endpoints
Bitgen et al., 2016 [43]	Prolactinoma (pituitary adenoma)	MN, NPBs, NBUDs, apoptosis and necrosis	Prolactin levels and pituitary adenoma diameters
Pardini et al., 2017 [21]	Bladder cancer	MN and NBUDs	-
Gerić et al., 2015 [44]	Papillary thyroid cancer	MN, NPBs, and NBUDs	-
Kiraz et al., 2016 [45]	Endometrial precancerous lesions and endometrial cancer	MN and NDI	Neoplastic and the pre-neoplastic conditions
Wu et al., 2016 [46]	Breast cancer	MN, NPBs, and NBUDs, apoptosis and necrosis	Vitamin B6 levels
Lombardi et al., 2015 [2]	Inflammatory bowel disease and polyps	MN in mononucleated cells in subjects with polyps	-
Ionescu et al., 2015 [20]	Colorectal neoplastic lesions (hyperplastic polyps, adenomas, adenocarcinomas)	NDI	-
Gashi et al., 2018 [7]	Cervical intraepithelial lesions	MN, NPBs, and NBUDs	HPV infection
Karpman et al., 2018 [47]	Lung cancer	MN, NPBs, and NBUDs	-
Cuceu et al., 2018 [48•]	Hodgkin lymphoma	MN and NPBs	-
El-Zein et al., 2017 [49•]	Small cell lung cancer and non-small cell lung cancer	MN, NPBs, and NBUDs	-
İpek et al., [16]	Coronary artery disease	MN and NDI	Coronary atherosclerosis severity indices SYNTAX and Gensini
Prasad et al., 2015 [50]	Diabetes	MN, NPBs, and NBUDs	Neuropathy
Salimi et al., 2016 [51]	Diabetes, diabetic nephropathy, and nephropathy	MN	-
Karaman et al., 2015 [52]	Metabolic syndrome	MN	Waist circumference, body-mass index, and plasma triglyceride levels
Guido et al., 2016 [53]	Chronic renal failure	MN	-
Donmez-Altuntas et al., 2017 [54]	Multinodular goiter	MN, apoptosis, and necrosis	Plasma 8-hydroxy-2′ -deoxyguanosine (marker of oxidative stress)
François et al., 2016 [55]	Mild cognitive impairment	MN and NBUDs	-
Xavier et al., 2017 [56]	Non-syndromic cleft lip and/ or palate	MN, NBUDs, and NPBs	-
Ferro et al., 2017 [57]	β-Thalassemia	MN	Serum ferritin
Francies et al., 2017 [58]	Fanconi anemia	MN	-
Šošić et al., 2017 [59]	Thrombophilia during pregnancy	MN	-
Coppedè et al., 2016 [60]	Young mothers of Down syndrome individuals	MN	-

Abbreviations: *MN* micronuclei, *NPBs* nucleoplasmic bridges, *NBUDs* nuclear buds, *NDI* nuclear division index

cardiovascular, metabolic, respiratory, and neurodegenerative ones) was carried out by Milic et al. [39•]. Increased levels of MN in PBL were shown in all of these disease groups, and thus could play a role in disease development and progression. In the literature published since 2015, previously obtained data were basically confirmed. A study of cardiovascular patients showed that as the degree of atherosclerosis increases and coronary flow worsens MN frequency in PBL increases and NDI decreases [16]. In diabetes, the oxidative stress is augmented leading to DNA damage, which is potentially linked to diabetic complications. CBMN endpoints are significantly higher in diabetes patient, both with and without neuropathy, than in controls. Patients with neuropathy demonstrated higher frequency of nuclear aberrations as compared

to the group without neuropathy [50]. MN level was significantly higher in diabetes, diabetic nephropathy, and nephropathy patients with no sign of diabetes, compared with controls. These results indicate that increased genomic instability expressed as MN is associated with nephropathy [51]. Both studies [50, 51] concluded that the implementation of CBMN assay at the clinical level would greatly enhance diagnosis, care, and management of diabetes patients. MN frequency was also significantly increased in patients with metabolic syndrome highlighting the value of this assay as biomarker [52].

Both, chronic renal failure and the dialysis used to treat chronic renal failure can contribute to chromosomal and/or genomic damage and increase of MN [53, 63]. Overall, MN frequency has the potential to predict chronic kidney disease associated complications [64].

Increased levels of CBMN endpoints and oxidative stress among patients with multinodular goiter (enlarged thyroid) may predict an increased risk of thyroid cancer [54].

An increase in lymphocyte CBMNCyt biomarkers may be associated with cognitive decline, as well and may be involved in the early development of Alzheimer's disease [55].

CIN, including MN formation, is a significant factor in development of the non-syndromic cleft lip and/or palate (NSCL/P) [56]. Levels of MN were significantly higher in transfusion-dependent β-thalassemic patients so regular monitoring of genomic instability can reduce risk of malignance, and/or lead to faster diagnoses [57]. The spontaneously occurring MN rates of the Fanconi anemia (FA) patients are significantly higher compared to the control group, indicating genomic instability. Ionizing irradiation- or mitomycin C-induced MN in lymphocytes of FA patients were also higher than in FA parents and controls. In mitomycin C-induced MN frequencies, a clear distinction between FA homozygotes, FA heterozygotes, and controls was observed. Thus, MN could be useful biomarker in epidemiological studies to distinguish populations that are more sensitive to genotoxic agents [58]. Pregnant women with thrombophilia have more chance of having a higher frequency of MN than pregnant women with no thrombophilia, probably because of oxidative stress initiated by prothrombotic condition [59].

MN test can also find its practical applications in perinatology. Despite that advanced maternal age represents the major risk factor for the birth of a child with Down syndrome (DS), most of DS babies are born nowadays from young women aging less than 35 years. An increased frequency of MN, shorter telomeres and impaired global DNA methylation were found in PBL of young mothers of DS individuals. The frequency of micronucleated lymphocytes correlated with methylenetetrahydrofolate reductase promoter methylation levels [60]. The baseline frequency for CBMNCyt markers decreased at 3 and 6 months relative to values measured in cord blood at birth. The study did not find correlation between mother's lifestyle characteristics (smoking, alcohol intake, folic acid consumption) and CBMN biomarkers measured in cord blood. Authors [65] provided baseline data on MN levels for further studies of DNA integrity and stability at the earliest phase of life and changes in DNA damage in the human life cycle.

In conclusion, chromosome damage and cytotoxic effects measured in PBL with application of CBMNCyt could be used to improve individual risk assessment, disease characterization, and more precise therapeutic intervention. Assuming that mechanisms of DNA damage and repair are similar in different tissues, peripheral lymphocytes can serve as an excellent marker, because of their short half-life and wide presence in the body [39•]. DNA damage and chromosomal alterations in PBL go together with DNA mutations in tumor tissues of different origin, and can be an informative biomarker not only of hematological but also of any other diseases. MN arise during precancerous and cancerous conditions and therefore are a valuable predictive indicator. Identifying this high-risk sub-group would have value for developing interventions and disease prevention. Application of molecular cytogenetic (FISH) techniques together with MN test will permit to precise genomic regions involved in CIN. The results accumulated so far substantiate the idea of introducing MN test as biomarkers into the clinical decision process [39•].

Micronuclei in Buccal Cells, Diagnostic, and Prognostic Significance

The study of DNA damage in exfoliated cells collected from the oral cavity holds great promise as a minimally invasive method. Buccal cells constitute the first point-of-contact for the inhalation or ingestion route, and are capable of metabolizing carcinogens to reactive products. MN in buccal cells are a valid method for the detection of cancer risk in humans, as the majority of tumors derive from epithelial. With respect to oral mucosa, it has been described that MN are observed in the basal layer of epithelial tissues, and their presence can reflect genotoxic damage that occurred up to 14 days prior to sample collection [38]. Moreover, buccal cells have been shown to have limited DNA repair capacity compared to PBL, and therefore may more precisely reflect genomic instability [66].

Bolognesi et al. [67] reviewed clinical applications of the MN test in exfoliated buccal mucosa cells in patients with oral, head-and-neck, breast, bladder, and other cancers, oral premalignant or non-malignant diseases, diabetes, different chronic diseases as well as with AD and DS. Heterogeneity in study designs with different schemes of subject recruitment and experimental protocols complicated the comparative analysis of available data. Our review here provides an update on the clinical application of the MN test in buccal cells since

2015. Accordingly MN evaluation in buccal cells allows for the characterizing of oral cavity diseases.

An association of MN presence in buccal cells and smoking was analyzed in a systematic review of de Geus et al. [66]. Despite the high variation in the methodology of the assessed studies, enhanced BMNCyt endpoints of smokers compared to nonsmokers was demonstrated. This confirmed the association of the tobacco-exposure with cytotoxic and genotoxic effects in buccal cells, that increase the cancer risk. It is noteworthy that the review of de Geus et al. [66] includes publications until 2014; after 2014, MN studies among smokers decreased; instead more analyses of other genotoxic risk factors were done by MN test (Table 2). Increase of MN number in buccal cells of individuals using various tobacco forms (smokeless tobacco and smoking tobacco) with potentially malignant oral diseases (leukoplakia, oral submucous fibrosis, lichen planus), oral squamous cell carcinoma (OSCC) and oral submucous fibrosis (OSMF) was shown [68–70].

MN frequency increased in potentially malignant, OSCC and OSMF groups compared to control and therefore can be considered as marker of epithelial carcinogenic progression. Similar results were obtained by Kohli et al. [71] who compared MN frequency in normal mucosa, in individuals using various tobacco forms without oral leukoplakia, and areca nut chewers with OSMF.

In addition to diseases of the oral cavity, the increase of BMNCyt endpoints was also reported for various diseases with pathology of other tissues. Increase of BMNCyt endpoints in breast cancer patients with smoking habit was more frequent before and during therapies compared to control [72]. In children with chronic kidney disease, elevation of BMNCyt markers was observed in the pre-dialysis stage, on regular hemodialysis and after transplantation [22]. These results were confirmed in another study indicating that MN frequency can increase due to the disease-state/dialysis/drug therapy [23].

Statistically significant increase in BMNCyt parameters was observed in sickle cell anemia patients compared with controls [73]. Concentration of plasma vitamin B12 in AD cases showed a positive correlation with number of MN and basal cells, while plasma homocysteine showed a negative correlation with karyorrhectic cells which may be explained by lower vitamin B12 and higher homocysteine levels, respectively [24]. Increase of nuclear abnormalities (NAs: micronucleated cells, binucleated cells, pyknotic nuclei, karyorrhexis, karyolysis, abnormally condensed chromatin, and NBUDs) in buccal cells were observed in patients with controlled or uncontrolled diabetes mellitus (DM) type I or II. BMNCyt parameters were

Table 2 BMNCyt endpoints in buccal cells of patients reported in publications of 2015–2018

References	Diseases	BMNCyt endpoints associated with the disease	Clinically relevant markers associated with BMNCyt endpoints
Dosi et al., 2016 [68]	Oral leukoplakia	MN	-
Sangle et al., 2016 [69]	Leukoplakia, oral submucous fibrosis, and lichen planus	MN	-
Shah et al., 2015 [70]	Oral submucous fibrosis, oral squamous cell carcinoma	MN	-
Kohli et al., 2017 [71]	Oral leukoplakia, oral submucous fibrosis	MN	-
Paz et al., 2018 [72]	Breast cancer	Karyorrhexis and karyolysis	-
Aykanat et al., 2016 [22]	Chronic kidney disease	MN, binucleated, and condensed chromatin cells	Pre-dialysis stage, regular haemodialysis, post-transplantation
Gandhi et al., 2017 [23]	Renal disease	MN, binucleated, and pyknotic cells	Disease state, dialysis, drug therapy
Naga et al., 2016 [73]	Sickle cell anemia	MN, binucleated cells, pyknosis, and karyolysis	-
Thomas and Fenech, 2015 [24]	Alzheimer's disease	Basal, condensed chromatin, and karyorrhectic cells	Plasma vitamin B and homocysteine
Gómez-Meda et al., 2016 [74]	Type I or II diabetes mellitus	MN, binucleated cells, pyknosis, karyorrhexis, karyolysis, abnormally condensed chromatin, and NBUDs	Folic acid deficiency

Abbreviations: *MN* micronuclei, *NBUDs* nuclear buds

significantly reduced after folic acid (antioxidant) supplementation confirming the idea that free radicals are responsible for the increased frequency of NAs in DM patients [74].

In conclusion, the literature reviewed represents diagnostic importance of MN scoring in buccal cells in clinical pathology. MN detection is an important step in the field of cancer prevention and therapeutics. In some cases of chronic diseases elevated levels of MN can be considered as indicator of higher risk for cancer development. Hence, MN are important biomarkers with huge potential in screening and predicting patients with oral (potentially) malignant disorders and also can act as risk assessors in patient's ongoing treatment for cancer. The increase in MN frequencies from normal mucosa to potentially malignant disorders to oral and non-oral cancer suggests a link of this biomarker with malignant neoplastic progression. The assay also has applications in non-target-tissue disease-monitoring.

Comparison of Micronuclei Results in Peripheral Blood Lymphocytes and Buccal Cells

For the first time, MN assay was simultaneously used to detect baseline genetic damages both in lymphocytes and buccal cells in patients having cervical lesions. MN in PBL and buccal cells, and NPB and NBUD in PBLs only, were increased in high-grade squamous intraepithelial lesions and squamous cervical cancer patients. Overall, MN frequency in buccal cells was correlated positively with MN frequency in PBL [7]. Older adults with frailty syndrome had significantly higher frequencies of MN in lymphocytes and of binucleated buccal cells, and lower frequencies of pyknotic and condensed chromatin buccal cells, than non-frail subjects. Similar results were obtained on cognitive status. Moreover, presence of frailty and cognitive impairment were independently related to increases in frequencies of PBL-associated MN and binucleated buccal cells. Thus, MN frequencies in lymphocytes were positively correlated with binucleated buccal cells [75].

A review [38] provided information about MN frequencies in autoimmune diseases (ADi) in lymphocytes, buccal mucosa, and fibroblasts. MN frequency was evaluated in different pathological conditions, including hyperthyroidism, diabetes mellitus, multiple sclerosis, vitiligo, psoriasis vulgaris, rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, and others. The increased level of MN was revealed in all ADi cases that were included in this review, even though it is not known if this is cause or consequence of the disease. MN frequencies tend to be higher in systemic ADi when compared to local affections. The presence of inflammatory cytokines, reactive oxygen species, and nitric oxide can be involved in the pathology of ADi as well as in the generation of MN. MN frequencies tended to be higher in lymphocytes than in buccal mucosa cells, irrespective of the disease evaluated. Furthermore, PBL-associated MN frequencies have high variability in ADi and healthy subjects.

Micronuclei in Cells of Different Origin, Diagnostic and Prognostic Significance

Only in few studies the MN levels were also evaluated in cells of other origin than PBL and buccal cells; however, such studies are limited by ethical considerations and possibility of material acquisition, e.g., during surgeries.

There was a gradual increase in MN scores in epithelial cells on breast cytology smears from benign to malignant category [13]. Statistically significant differences were found in the number of micronucleated cervical cells between patients with papillomavirus infection and healthy women. Moreover, significant associations were found between MN expression and both the degree of uterine lesions and viral load [12]. MN scores in cervical cytology smears were significantly different between patients with endometrial carcinoma, atypical and benign cells [76]. A statistically significant difference between the presence of MN and karyorrhexis was shown in exfoliated cervical epithelial cells of *Trichomonas vaginalis*-infected group [77]. MN in cervical exfoliated cell smears was significantly higher in high-grade squamous intraepithelial lesion and invasive carcinoma cases compared to low-grade squamous intraepithelial lesion and non-neoplastic cases [78]. The risk of cervix cancer can be assessed in urothelial cells isolated from urine (if specimen appropriately handled cells can be stored up to 24 h), which is much easier to obtain than blood or cervix epithelium samples. A statistically significant difference in MN levels was found in patients with normal cervix and cervix erosion, hypertrophy, or abnormal growth [79]. It was shown that the number of micronucleated cells in pleural effusion cytology samples was significantly higher in patients with malignant outcome compared to cases with benign outcome [80].

While CIN in somatic cells may be a predisposing factor for neoplasia in germ cells, it may affect fertility. In fact, one of the main reasons for the limited success of in vitro fertilization are chromosomal abnormalities. Carbone and Chavez [81] discussed pre-implantation chromosomal instability in embryos, including MN formation, and its potential translation to clinical applications in reproductive medicine. Daughtry and Chavez [82] reported findings of MN in cleavage-stage human embryos and confirmed that identification of aneuploid embryos is actual task of pre-implantation genetic screening.

In conclusion, the possibility of MN test application in tissues of various origins allows evaluating CIN in targets of pathological processes, which is of particular interest from the

point of view of application in practical medicine. Tissue samples can be taken during medical procedures and the results of these analyses can complement medical data.

Micronuclei and Chromothripsis

Chromothripsis is a recently described "chromosome catastrophe" phenomenon in which multiple genomic rearrangements are generated in a single catastrophic event. A model correlating MN formation with chromosome pulverization was proposed by Crasta et al. [25••] and confirmed by Zhang et al. [26••]. Crasta et al. [25••] studied nocodazole-induced formation of whole-chromosome containing MN in vitro. These MN undergo defective and asynchronous DNA replication, resulting in DNA damage and often extensive fragmentation of the chromosome. Pulverization of chromosomes in MN may be one of explanations for chromothripsis in cancer and developmental disorders, where isolated in MN chromosomes undergo massive local DNA breakage and rearrangement. Chromosomes within MN can reincorporate into daughter nuclei following mitosis, the remaining MN persisted in cells well into the second generation. Thus, mutations, being present in MN, can be incorporated into a stably transmitted genome. Zhang et al. [26••] demonstrated by a combination of live cell imaging and single-cell genome sequencing, that MN formation can indeed generate a spectrum of complex genomic rearrangements. Authors proposed that the physical isolation of chromosomes in MN might explain the localization of DNA lesions in chromothripsis. The traditional conception of MN formation thus has at least partially been overturned. MN have evolved from passive indicators of DNA damage to active players in the formation of DNA lesions, thus unraveling previously unforeseen roles of MN in the origins of CIN in tumors [83–85]. As chromothripsis has most frequently been associated with cancer [86, 87] and less often described in patients with developmental disorders and congenital anomalies [88, 89], there is no direct evidence, but it is possible that also in the latter cases chromothrypsis could arise due to the formation of MN.

Conclusions

Review of publications for 2015–2018 indicates a high sensitivity of MN assay in PBL, buccal, and other cells for evaluation of genotoxic, cytotoxic, and cytostatic effects associated with different diseases. Almost all studies show an increase in the levels of MN and/or nuclear anomalies in patients with different pathologies. The results presented indicate that the MN assay is a sensitive prognostic and diagnostic biomarker. One of the main problems of using MN test in clinics are the variability of the results and the complexity of taking into account the effects of endogenous and exogenous confounding factors. The cause of increased level of CIN in patients often remains unclear; only in some cases it can be associated with oxidative stress. It is not always clear whether an increase in MN is the cause or consequence of pathological processes in the organism. There are pathologies in which an increase in MN is shown in both leukocytes and buccal cells (diabetes, breast cancer, renal diseases). Thus, the MN test demonstrated that CIN can develop in different tissues, and not only in disease targets. Recently identified relationships between MN and chromothripsis increased significantly the importance of MN research in pathologies.

Funding This study was funded by RA MES SCS, within the frames of projects #A/G-17 and RA MES SCS–YSU–RF SFU No. 4.

Compliance with Ethical Standards

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

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Kotze MJ, Lückhoff HK, Peeters AV, Baatjes K, Schoeman M, van der Merwe L, et al. Genomic medicine and risk prediction across the disease spectrum. Crit Rev Clin Lab Sci. 2015;52:120–37. https://doi.org/10.3109/10408363.2014.997930.
2. Lombardi S, Fuoco I, di Fluri G, Costa F, Ricchiuti A, Biondi G, et al. Genomic instability and cellular stress in organ biopsies and peripheral blood lymphocytes from patients with colorectal cancer and predisposing pathologies. Oncotarget. 2015;6:14852–64.
3. Barzilai A, Schumacher B, Shiloh Y. Genome instability: linking ageing and brain degeneration. Mech Ageing Dev. 2017;161:4–18. https://doi.org/10.1016/j.mad.2016.03.011.
4. Nikolouzakis TK, Vassilopoulou L, Fragkiadaki P, Mariolis Sapsakos T, Papadakis GZ, Spandidos DA, et al. Improving diagnosis, prognosis and prediction by using biomarkers in CRC patients (review). Oncol Rep. 2018;39:2455–72. https://doi.org/10.3892/or.2018.6330.
5. Schrodi SJ, Mukherjee S, Shan Y, Tromp G, Sninsky JJ, Callear AP, et al. Genetic-based prediction of disease traits: prediction is very difficult, especially about the future. Front Genet. 2014;5:162. https://doi.org/10.3389/fgene.2014.00162.
6. Rysz J, Gluba-Brzózka A, Franczyk B, Jabłonowski Z, Ciałkowska-Rysz A. Novel biomarkers in the diagnosis of chronic

kidney disease and the prediction of its outcome. Int J Mol Sci. 2017;18:E1702. https://doi.org/10.3390/ijms18081702.
7. Gashi G, Mahovlić V, Manxhuka-Kerliu S, Podrimaj-Bytyqi A, Gashi L, Elezaj IR. The association between micronucleus, nucleoplasmic bridges, and nuclear buds frequency and the degree of uterine cervical lesions. Biomarkers. 2018;23:364–72. https://doi.org/10.1080/1354750X.2018.1428828.
8. Fenech M, Kirsch-Volders M, Natarajan AT, Surralles J, Crott JW, Parry J, et al. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis. 2011;26:125–32. https://doi.org/10.1093/mutage/geq052.
9. Torres-Bugarín O, Zavala-Cerna MG, Nava A, Flores-García A, Ramos-Ibarra ML. Potential uses, limitations, and basic procedures of micronuclei and nuclear abnormalities in buccal cells. Dis Markers. 2014;2014:956835. https://doi.org/10.1155/2014/956835.
10. Sabharwal R, Verma P, Syed MA, Sharma T, Subudhi SK, Mohanty S, et al. Emergence of micronuclei as a genomic biomarker. Indian J Med Paediatr Oncol. 2015;36:212–8. https://doi.org/10.4103/0971-5851.171541.
11. Bolognesi C, Fenech M. Micronucleus assay in human cells: lymphocytes and buccal cells. Methods Mol Biol. 2013;1044:191–207. https://doi.org/10.1007/978-1-62703-529-3_10.
12. Adam ML, Pini C, Túlio S, Cantalice JC, Torres RA, Dos Santos Correia MT. Assessment of the association between micronuclei and the degree of uterine lesions and viral load in women with human papillomavirus. Cancer Genomics Proteomics. 2015;12:67–71.
13. Sylvia MT, Baskaran L, Bhat RV. Micronucleus study on breast cytology aspirate smears and its diagnostic utility. J Cytol. 2018;35:22–6. https://doi.org/10.4103/JOC.JOC_160_16.
14. El-Zein R, Vral A, Etzel CJ. Cytokinesis-blocked micronucleus assay and cancer risk assessment. Mutagenesis. 2011;26:101–6. https://doi.org/10.1093/mutage/geq071.
15. El-Zein RA, Lopez MS, D'Amelio AM Jr, Liu M, Munden RF, Christiani D, et al. The cytokinesis-blocked micronucleus assay as a strong predictor of lung cancer: extension of a lung cancer risk prediction model. Cancer Epidemiol Biomark Prev. 2014;23:2462–70. https://doi.org/10.1158/1055-9965.EPI-14-0462.
16. İpek E, Ermiş E, Uysal H, Kızılet H, Demirelli S, Yıldırım E, et al. The relationship of micronucleus frequency and nuclear division index with coronary artery disease SYNTAX and Gensini scores. Anatol J Cardiol. 2017;17:483–9. https://doi.org/10.14744/AnatolJCardiol.2017.7582.
17. Thomas P, Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, et al. Buccal micronucleus cytome assay. Nat Protoc. 2009;4:825–37. https://doi.org/10.1038/nprot.2009.53.
18. Thomas P, Fenech M. Buccal micronucleus cytome assay. Methods Mol Biol. 2011;682:235–48. https://doi.org/10.1007/978-1-60327-409-8_17.
19. Kashyap B, Reddy PS. Micronuclei assay of exfoliated oral buccal cells: means to assess the nuclear abnormalities in different diseases. J Cancer Res Ther. 2012;8:184–91. https://doi.org/10.4103/0973-1482.98968.
20. Ionescu EM, Nicolaie T, Ionescu MA, Becheanu G, Andrei F, Diculescu M, et al. Predictive cytogenetic biomarkers for colorectal neoplasia in medium risk patients. J Med Life. 2015;8:398–403.
21. Pardini B, Viberti C, Naccarati A, Allione A, Oderda M, Critelli R, et al. Increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of bladder cancer. Br J Cancer. 2017;116:202–10. https://doi.org/10.1038/bjc.2016.411.
22. Aykanat B, Demircigil GC, Buyan N, Baskin E, Gulleroglu K, Fidan K, et al. Micronuclei and other nuclear anomalies in buccal epithelial cells of children with chronic kidney disease. Arh Hig Rada Toksikol. 2016;67:317–25. https://doi.org/10.1515/aiht-2016-67-2851.
23. Gandhi G, Tung G. Sensitivity and specificity prediction of the buccal micronucleus cytome assay in end-stage renal disease patients on dialysis: a case-control study. Mutat Res. 2017;822:1–9. https://doi.org/10.1016/j.mrgentox.2017.07.001.
24. Thomas P, Fenech M. Buccal cytome biomarkers and their association with plasma folate, vitamin B12 and homocysteine in Alzheimer's disease. J Nutrigenet Nutrigenomics. 2015;8:57–69. https://doi.org/10.1159/000435784.
25.•• Crasta K, Ganem NJ, Dagher R, Lantermann AB, Ivanova EV, Pan Y, et al. DNA breaks and chromosome pulverization from errors in mitosis. Nature. 2012;482:53–8. https://doi.org/10.1038/nature10802 **This study was among the first to demonstrate that chromothripsis can originate from DNA damage in micronuclei.**
26.•• Zhang CZ, Spektor A, Cornils H, Francis JM, Jackson EK, Liu S, et al. Chromothripsis from DNA damage in micronuclei. Nature. 2015;522:179–84. https://doi.org/10.1038/nature14493 **This study was among the first to demonstrate that chromothripsis can originate from DNA damage in micronuclei.**
27. Fenech M, Morley AA. Measurement of micronuclei in lymphocytes. Mutat Res. 1985;147:29–36.
28.• Hintzsche H, Hemmann U, Poth A, Utesch D, Lott J, Stopper H. Fate of micronuclei and micronucleated cells. Mutat Res. 2017;771:85–98. https://doi.org/10.1016/j.mrrev.2017.02.002 **This article presents the first comprehensive data on the fate of micronuclei in cell generations.**
29. Corvi R, Albertini S, Hartung T, Hoffmann S, Maurici D, Pfuhler S, et al. ECVAM retrospective validation of in vitro micronucleus test (MNT). Mutagenesis. 2008;23:271–83. https://doi.org/10.1093/mutage/gen010.
30. Homiski ML, Muehlbauer PA, Dobo KL, Schuler MJ, Aubrecht J. Concordance analysis of an in vitro micronucleus screening assay and the regulatory chromosome aberration assay using pharmaceutical drug candidates. Environ Mol Mutagen. 2010;51:39–47. https://doi.org/10.1002/em.20507.
31. Fenech M. Cytokinesis-block micronucleus cytome assay. Nat Protoc. 2007;2:1084–104.
32. Bolognesi C, Knasmueller S, Nersesyan A, Roggieri P, Ceppi M, Bruzzone M, et al. Inter-laboratory consistency and variability in the buccal micronucleus cytome assay depends on biomarker scored and laboratory experience: results from the HUMNxl international inter-laboratory scoring exercise. Mutagenesis. 2017;32(2):257–66. https://doi.org/10.1093/mutage/gew047.
33. Gajski G, Gerić M, Oreščanin V, Garaj-Vrhovac V. Cytokinesis-block micronucleus cytome assay parameters in peripheral blood lymphocytes of the general population: contribution of age, sex, seasonal variations and lifestyle factors. Ecotoxicol Environ Saf. 2018;148:561–70. https://doi.org/10.1016/j.ecoenv.2017.11.003.
34. Fenech M, Holland N, Zeiger E, Chang WP, Burgaz S, Thomas P, et al. The HUMN and HUMNxL international collaboration projects on human micronucleus assays in lymphocytes and buccal cells–past, present and future. Mutagenesis. 2011;26:239–45. https://doi.org/10.1093/mutage/geq051.
35. Bonassi S, Coskun E, Ceppi M, Lando C, Bolognesi C, Burgaz S, et al. The HUman MicroNucleus project on eXfoLiated buccal cells (HUMN(XL)): the role of life-style, host factors, occupational exposures, health status, and assay protocol. Mutat Res. 2011;728:88–97. https://doi.org/10.1016/j.mrrev.2011.06.005.
36. Bonassi S, Znaor A, Ceppi M, Lando C, Chang WP, Holland N, et al. An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. Carcinogenesis. 2007;28:625–31.
37. Bonassi S, El-Zein R, Bolognesi C, Fenech M. Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence

from human studies. Mutagenesis. 2011;26:93–100. https://doi.org/10.1093/mutage/geq075.

38. Torres-Bugarín O, Macriz Romero N, Ramos Ibarra ML, Flores-García A, Valdez Aburto P, Zavala-Cerna MG. Genotoxic effect in autoimmune diseases evaluated by the micronucleus test assay: our experience and literature review. Biomed Res Int. 2015;2015:194031. https://doi.org/10.1155/2015/194031.

39.• Milic M, Frustaci A, Del Bufalo A, Sánchez-Alarcón J, Valencia-Quintana R, Russo P, et al. DNA damage in non-communicable diseases: A clinical and epidemiological perspective. Mutat Res. 2015;776:118–27. https://doi.org/10.1016/j.mrfmmm.2014.11.009 **This study demonstrates the importance of chromosomal instability measured by the MN test in non-communicable diseases.**

40. Vargas-Rondón N, Villegas VE, Rondón-Lagos M. The role of chromosomal instability in cancer and therapeutic responses. Cancers (Basel). 2017;10:E4. https://doi.org/10.3390/cancers10010004.

41. Fenech M. Cytokinesis-block micronucleus assay evolves into a "cytome" assay of chromosomal instability, mitotic dysfunction and cell death. Mutat Res. 2006;600:58–66.

42. Bhatia A, Kumar Y. Relevance of microscopic indicators of chromosomal instability in routine reporting of malignancies. Diagn Cytopathol. 2014;42:181–8. https://doi.org/10.1002/dc.23012.

43. Bitgen N, Donmez-Altuntas H, Bayram F, Cakir I, Hamurcu Z, Diri H, et al. Increased micronucleus, nucleoplasmic bridge, nuclear bud frequency and oxidative DNA damage associated with prolactin levels and pituitary adenoma diameters in patients with prolactinoma. Biotech Histochem. 2016;91:128–36. https://doi.org/10.3109/10520295.2015.1101163.

44. Gerić M, Janušić R, Šarčević B, Garaj-Vrhovac V. A case-control study of genotoxicity endpoints in patients with papillary thyroid cancer. Mutat Res Genet Toxicol Environ Mutagen. 2015;784-785:47–50. https://doi.org/10.1016/j.mrgentox.2015.05.006.

45. Kiraz A, Açmaz G, Uysal G, Unal D, Dönmez-Altuntas H. Micronucleus testing as a cancer detector: endometrial hyperplasia to carcinoma. Arch Gynecol Obstet. 2016;293:1065–71. https://doi.org/10.1007/s00404-015-3867-y.

46. Wu X, Xu W, Zhou T, Cao N, Ni J, Zou T, et al. The role of genetic polymorphisms as related to one-carbon metabolism, vitamin B6, and gene-nutrient interactions in maintaining genomic stability and cell viability in Chinese breast cancer patients. Int J Mol Sci. 2016;17:E1003. https://doi.org/10.3390/ijms17071003.

47. Karpman MD, Eldridge R, Follis JL, Etzel CJ, Shete S, El-Zein RA. Chronic obstructive pulmonary disease among lung cancer-free smokers: the importance of healthy controls. Respir Investig. 2018;56:28–33. https://doi.org/10.1016/j.resinv.2017.11.002.

48.• Cuceu C, Hempel WM, Sabatier L, Bosq J, Carde P, M'kacher R. Chromosomal instability in Hodgkin lymphoma: An in-depth review and perspectives. Cancers (Basel). 2018;10:E91. https://doi.org/10.3390/cancers10040091 **This is one of the few studies where MN test is applied together with the fluorescence in situ hybridization (FISH) to spread light on cytogenetic targets of diseases and anticancer therapy.**

49.• El-Zein RA, Abdel-Rahman S, Santee KJ, Yu R, Shete S. Identification of small and non-small cell lung cancer markers in peripheral blood using cytokinesis-blocked micronucleus and spectral karyotyping assays. Cytogenet Genome Res. 2017;152:122–31. https://doi.org/10.1159/000479809 **This is one of the few studies where MN test is applied together with the fluorescence in situ hybridization (FISH) to spread light on cytogenetic targets of diseases and anticancer therapy.**

50. Prasad M, Bronson SC, Warrier T, Badarinath A, Rai S, Baid K, et al. Evaluation of DNA damage in type 2 diabetes mellitus patients with and without peripheral neuropathy: a study in south Indian population. J Nat Sci Biol Med. 2015;6:80–4. https://doi.org/10.4103/0976-9668.149096.

51. Salimi M, Broumand B, Mozdarani H. Association of elevated frequency of micronuclei in peripheral blood lymphocytes of type 2 diabetes patients with nephropathy complications. Mutagenesis. 2016;31:627–33.

52. Karaman A, Aydın H, Geçkinli B, Çetinkaya A, Karaman S. DNA damage is increased in lymphocytes of patients with metabolic syndrome. Mutat Res Genet Toxicol Environ Mutagen. 2015;782:30–5. https://doi.org/10.1016/j.mrgentox.2015.03.009.

53. Guido M, Zizza A, Tumolo MR, Stefanelli G, D'Alba M, Idolo A, et al. Using the cytokinesis-block micronucleus cytome assay to evaluate chromosomal DNA damage in chronic renal patients undergoing bicarbonate haemodialysis and haemodiafiltration. J Prev Med Hyg. 2016;57:E178–84.

54. Donmez-Altuntas H, Bayram F, Bitgen N, Ata S, Hamurcu Z, Baskol G. Increased chromosomal and oxidative DNA damage in patients with multinodular goiter and their association with cancer. Int J Endocrinol. 2017;2017:2907281. https://doi.org/10.1155/2017/2907281.

55. François M, Leifert WR, Hecker J, Faunt J, Fenech MF. Guanine-quadruplexes are increased in mild cognitive impairment and correlate with cognitive function and chromosomal DNA damage. DNA Repair (Amst). 2016;46:29–36. https://doi.org/10.1016/j.dnarep.2016.08.001.

56. Xavier LA, Bezerra JF, de Rezende AA, Oliveira RA, Dalmolin RJ, do Amaral VS. Analysis of genome instability biomarkers in children with non-syndromic orofacial clefts. Mutagenesis. 2017;32:313–21. https://doi.org/10.1093/mutage/gew068.

57. Ferro E, Visalli G, La Rosa MA, Piraino B, Civa R, Randazzo Papa G, et al. Genotoxic effect of iron overload and disease complications in transfused β thalassaemic patients. Mutagenesis. 2017;32:275–81. https://doi.org/10.1093/mutage/gew062.

58. Francies FZ, Wainwright R, Poole J, De Leeneer K, Coene I, Wieme G, et al. Diagnosis of Fanconi anaemia by ionising radiation- or mitomycin C-induced micronuclei. DNA Repair (Amst). 2018;61:17–24. https://doi.org/10.1016/j.dnarep.2017.11.001.

59. Šošić GM, Jović N, Rakić B, Dimitrijević A, Varjačić M. Association between inherited thrombophilia in pregnancy and micronucleus frequency in peripheral blood lymphocytes. Balkan J Med Genet. 2017;20:11–8. https://doi.org/10.1515/bjmg-2017-0024.

60. Coppedè F, Denaro M, Tannorella P, Migliore L. Increased MTHFR promoter methylation in mothers of down syndrome individuals. Mutat Res. 2016;787:1–6. https://doi.org/10.1016/j.mrfmmm.2016.02.008.

61. Hovhannisyan G, Aroutiounian R, Liehr T. Chromosomal composition of micronuclei in human leukocytes exposed to mitomycin C. J Histochem Cytochem. 2012;60:316–22. https://doi.org/10.1369/0022155412436587.

62. Hovhannisyan G, Aroutiounian R, Babayan N, Harutyunyan T, Liehr T. Comparative analysis of individual chromosome involvement in micronuclei induced by mitomycin C and bleomycin in human leukocytes. Mol Cytogenet. 2016;9:49. https://doi.org/10.1186/s13039-016-0258-4.

63. Rodríguez-Ribera L, Pastor S, Corredor Z, Silva I, Diaz JM, Ballarin J, et al. Genetic damage in patients moving from hemodialysis to online hemodiafiltration. Mutagenesis. 2016;31:131–5. https://doi.org/10.1093/mutage/gev063.

64. Schupp N, Stopper H, Heidland A. DNA damage in chronic kidney disease: evaluation of clinical biomarkers. Oxidative Med Cell Longev. 2016;2016:3592042. https://doi.org/10.1155/2016/3592042.

65. Dass Singh M, Thomas P, Hor M, Almond T, Owens J, Hague W, et al. Infant birth outcomes are associated with DNA damage

biomarkers as measured by the cytokinesis block micronucleus cytome assay: the DADHI study. Mutagenesis. 2017;32:355–70. https://doi.org/10.1093/mutage/gex001.

66. de Geus JL, Wambier LM, Bortoluzzi MC, Loguercio AD, Kossatz S, Reis A. Does smoking habit increase the micronuclei frequency in the oral mucosa of adults compared to non-smokers? A systematic review and meta-analysis. Clin Oral Investig. 2018;22(1):81–91. https://doi.org/10.1007/s00784-017-2246-4.
67. Bolognesi C, Bonassi S, Knasmueller S, Fenech M, Bruzzone M, Lando C, et al. Clinical application of micronucleus test in exfoliated buccal cells: a systematic review and metanalysis. Mutat Res Rev Mutat Res. 2015;766:20–31. https://doi.org/10.1016/j.mrrev.2015.07.002.
68. Dosi T, Gupta D, Hazari A, Rajput R, Chauhan P, Rajapuri AS. Assessment of micronuclei frequency in individuals with a habit of tobacco by means of exfoliated oral buccal cells. J Int Soc Prev Community Dent. 2016;6:S143–7. https://doi.org/10.4103/2231-0762.189745.
69. Sangle VA, Bijjaragi S, Shah N, Kangane S, Ghule HM, Rani SA. Comparative study of frequency of micronuclei in normal, potentially malignant diseases and oral squamous cell carcinoma. J Nat Sci Biol Med. 2016;7:33–8. https://doi.org/10.4103/0976-9668.175049.
70. Shah SN, Manjunatha BS, Shah VS, Dagrus K, Soni N, Shah S. Quantitative evaluation of micronuclei in oral squamous cell carcinoma and oral submucous fibrosis patients: a comparative study. Recent Pat Anticancer Drug Discov. 2015;10:233–8.
71. Kohli M, Ahuja P, Mehendiratta M, Sharma M, Dutta J. Micronucleus assay: an early diagnostic tool to assess genotoxic changes in patients with tobacco use, oral leukoplakia and oral submucous fibrosis. J Clin Diagn Res. 2017;11:ZC28–32. https://doi.org/10.7860/JCDR/2017/27711.10567.
72. Paz MFCJ, de Alencar MVOB, Gomes Junior AL, da Conceição Machado K, Islam MT, Ali ES, et al. Correlations between risk factors for breast cancer and genetic instability in cancer patients. A clinical perspective study. Front Genet. 2018;8:236. https://doi.org/10.3389/fgene.2017.00236.
73. Naga MB, Gour S, Nallagutta N, Ealla KK, Velidandla S, Manikya S. Buccal micronucleus cytome assay in sickle cell disease. J Clin Diagn Res. 2016;10(6):ZC62–4. https://doi.org/10.7860/JCDR/2016/19984.7998.
74. Gómez-Meda BC, Zamora-Perez AL, Muñoz-Magallanes T, Sánchez-Parada MG, García Bañuelos JJ, Guerrero-Velázquez C, et al. Nuclear abnormalities in buccal mucosa cells of patients with type I and II diabetes treated with folic acid. Mutat Res Genet Toxicol Environ Mutagen. 2016;797:1–8. https://doi.org/10.1016/j.mrgentox.2015.12.003.
75. Sánchez-Flores M, Marcos-Pérez D, Lorenzo-López L, Maseda A, Millán-Calenti JC, Bonassi S, et al. Frailty syndrome and genomic instability in older adults. Suitability of the cytome micronucleus assay as a diagnostic tool. J Gerontol A Biol Sci Med Sci. 2018;73(7):864–72. https://doi.org/10.1093/gerona/glx258.
76. Gupta P, Dey P, Gupta N. Micronucleus scoring: a potential adjunct for diagnosis of endometrial adenocarcinoma on cervical samples. APMIS. 2015;123:930–4. https://doi.org/10.1111/apm.12441.
77. Safi Oz Z, Doğan Gun B, Gun MO, Ozdamar SO. Cytomorphometric and morphological analysis in women with trichomonas vaginalis infection: micronucleus frequency in exfoliated cervical epithelial cells. Acta Cytol. 2015;59:258–64. https://doi.org/10.1159/000431148.
78. Shi YH, Wang BW, Tuokan T, Li QZ, Zhang YJ. Association between micronucleus frequency and cervical intraepithelial neoplasia grade in thinprep cytological test and its significance. Int J Clin Exp Pathol. 2015;8:8426–32.
79. Sundararajan SK, Natarajan PS. Kanchana. Micronucleus assay in urothelial cells in cancer cervix. J Clin Diagn Res. 2017;11:XC01–3. https://doi.org/10.7860/JCDR/2017/23425.9488.
80. Kökenek Ünal TD, Çoban İ. Increased micronucleus count predicts malignant behavior in pleural effusion fluid. Turk J Med Sci. 2018;48:354–60. https://doi.org/10.3906/sag-1711-63.
81. Carbone L, Chavez SL. Mammalian pre-implantation chromosomal instability: species comparison, evolutionary considerations, and pathological correlations. Syst Biol Reprod Med. 2015;61:321–35. https://doi.org/10.3109/19396368.2015.1073406.
82. Daughtry BL, Chavez SL. Chromosomal instability in mammalian pre-implantation embryos: potential causes, detection methods, and clinical consequences. Cell Tissue Res. 2016;363:201–25. https://doi.org/10.1007/s00441-015-2305-6.
83. Terradas M, Martín M, Genescà A. Impaired nuclear functions in micronuclei results in genome instability and chromothripsis. Arch Toxicol. 2016;90:2657–67. https://doi.org/10.1007/s00204-016-1818-4.
84. Terradas M, Martín M, Genescà A. Detection of impaired DNA replication and repair in micronuclei as indicators of genomic instability and chromothripsis. Methods Mol Biol. 1769;2018:197–208. https://doi.org/10.1007/978-1-4939-7780-2_13.
85. Ly P, Cleveland DW. Rebuilding chromosomes after catastrophe: emerging mechanisms of chromothripsis. Trends Cell Biol. 2017;27:917–30. https://doi.org/10.1016/j.tcb.2017.08.005.
86. Smetana J, Oppelt J, Štork M, Pour L, Kuglík P. Chromothripsis 18 in multiple myeloma patient with rapid extramedullary relapse. Mol Cytogenet. 2018;11:7. https://doi.org/10.1186/s13039-018-0357-5.
87. van Poppelen NM, Yavuzyigitoglu S, Smit KN, Vaarwater J, Eussen B, Brands T, et al. Chromosomal rearrangements in uveal melanoma: Chromothripsis. Genes Chromosomes Cancer. 2018;57(9):452–8. https://doi.org/10.1002/gcc.4.
88. Anderson SE, Kamath A, Pilz DT, Morgan SM. A rare example of germ-line chromothripsis resulting in large genomic imbalance. Clin Dysmorphol. 2016;25:58–62. https://doi.org/10.1097/MCD.0000000000000113.
89. Gamba BF, Richieri-Costa A, Costa S, Rosenberg C, Ribeiro-Bicudo LA. Chromothripsis with at least 12 breaks at 1p36.33-p35.3 in a boy with multiple congenital anomalies. Mol Gen Genomics. 2015;290:2213–6. https://doi.org/10.1007/s00438-015-1072-0.