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# "Micronuclei and Disease" special issue: Aims, scope, and synthesis of outcomes

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## ABSTRACT

The purpose of the "Micronuclei and Disease" special issue (SI) is to: (i) Determine the level of evidence for association of micronuclei (MN), a biomarker of numerical and structural chromosomal aberrations, with risk of specific diseases in humans; (ii) Define plausible mechanisms that explain association of MN with each disease; (iii) Identify knowledge gaps and research needed to translate MN assays into clinical practice.

The "MN and Disease" SI includes 14 papers. The first is a review of mechanisms of MN formation and their consequences in humans. 11 papers are systematic reviews and/or meta-analyses of the association of MN with reproduction, child health, inflammation, auto-immune disease, glycation, metabolic diseases, chronic kidney disease, cardiovascular disease, eleven common cancers, ageing and frailty. The penultimate paper focuses on effect of interventions on MN frequency in the elderly. A road map for translation of MN data into clinical practice is the topic of the final paper.

The majority of reviewed studies were case-control studies in which the ratio of mean MN frequency in disease cases relative to controls, i.e. the mean ratio (MR), was calculated. The mean of these MR values, estimated by meta-analyses, for lymphocyte and buccal cell MN in non-cancer diseases were 2.3 and 3.6 respectively, and for cancers they were 1.7 and 2.6 respectively. The highest MR values were observed in studies of cancer cases in which MN were measured in the same tissue as the tumour (MR = 4.9–10.8).

This special issue is an important milestone in the evidence supporting MN as a reliable genomic biomarker of developmental and degenerative disease risk. These advances, together with results from prospective cohort studies, are helping to identify diseases in which MN assays can be practically employed in the clinical setting to better identify high risk patients and to prioritise them for preventive therapy.

*Abbreviations:* AGEs, advanced glycation end products; BMI, body mass index; BUC, buccal; CAD, coronary artery disease; CBMN, cytokinesis-block micronucleus; CDC, cervix derived cells; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disorder; cGAS-STING, cyclic GMP-AMP Synthase-Stimulator of Interferon Genes; HbA1c, glycated haemoglobin; IBD, inflammatory bowel disease; LYM, lymphocyte; MN, micronuclei; MR, mean ratio; NPB, nucleoplasmic bridges; NBUD, nuclear buds; RBC, red blood cell; RCT, randomised controlled trial; SEM, standard error of the mean; UDC, urine derived cells.

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## 1. Introduction

Micronuclei (MN) are produced in cells from malsegregation of chromosome fragments and/or whole chromosomes during mitosis and are considered to be one of the best validated biomarkers of chromosome instability [1]. They are also indicators of defective DNA replication and repair, mitotic errors caused by genetic defects, exposure to environmental or endogenous genotoxins, nutritional deficiency or excess, and genotoxic life-style factors.

Furthermore, MN increase with aging and are associated cross-sectionally and prospectively with infertility, pregnancy complications, developmental defects, obesity, diabetes, kidney disease, cardiovascular disease, a wide-range of cancers, cognitive impairment and neurological disorders such as Parkinson's disease and Alzheimer's disease [2]. The frequency of MN is modifiable by appropriate diet and life-style intervention and could prove to be an important biomarker for monitoring the efficacy and safety of such interventions aimed at improving health at the fundamental genome level [3].

Other cytogenetic biomarkers associated with MN are nucleoplasmic bridges (NPB) which originate from dicentric chromosomes caused by mis-repair of DNA breaks or telomere end fusions, and nuclear buds (NBUD) which originate from extrusion of amplified DNA and unresolved DNA repair complexes [1,4].

The purpose of the "Micronuclei and Disease" special issue is as follows:

- Determine the current level of evidence for association of MN, NPB and NBUD with risk of each specific disease including its progression, prognosis and genotoxic consequences of therapy and/or its efficacy.
- Define plausible mechanisms of MN formation that may explain association with each disease and evidence that may support it.
- Identify knowledge gaps and further work needed to justify translating use of MN, NPB or NBUD measurements into clinical practice.

It was anticipated that the great majority of data would relate only to MN because the adoption of the NPB and NBUD biomarkers occurred more than two decades after the lymphocyte cytokinesis block micronucleus (CBMN) assay was first reported in 1984. As a consequence, this brief overview is mainly focused on the outcomes relating to MN assays.

Key aspects to be tackled by each review included:

- A brief description of the purpose of the review and biological plausibility of association of MN, NPB or NBUD with the specific disease investigated
- Narrative review of case-control studies including a summary description of all studies in a Table. This table should include information on number of case and control subjects, outcome measure data (MN, NPB, NBUD) expressed as Mean (+/- SEM) in cases and controls, Mean Ratio (MR) of MN, NPB and NBUD biomarker data of cases relative to controls, including 95 % confidence intervals and P values.
- Prospective studies (if available) are described separately from case-control studies in an additional section.
- Meta-analysis of published data (assuming sufficient studies are available and heterogeneity is not too high)
- Assessment of the potential clinical utility (e.g. triage of patients as low/high risk, prognosis, susceptibility to drug genotoxicity etc…) and knowledge gaps to translate use of MN, NPB or NBUD into clinical practice.

## 2. Synopsis and outcomes of individual reviews

The "MN and Disease" SI includes fourteen papers in addition to this one. The first is a review on mechanisms of MN formation and their consequences in humans. The following eleven papers are narrative or systematic reviews and/or meta-analyses of the association of MN with reproduction and child health, inflammation and auto-immune diseases, glycation and metabolic diseases, chronic kidney disease, cardiovascular disease, eleven different common cancers, ageing and frailty. The penultimate paper focuses on the impact of interventions on MN frequency in the elderly. A road map for the translation of MN frequency data into clinical practice is the topic of the final paper. Highlights from these papers are described below.

The mechanisms review of Fenech et al. [5] describes the biology of the best validated MN assays used in humans namely the lymphocyte cytokinesis-block micronucleus (CBMN) cytome assay, the buccal cell MN cytome assay and the red blood cell MN assay. Apart from the well-established mechanisms of MN formation the review highlighted the two most relevant recent discoveries that entrapment of a chromosome within a MN leads to two fundamental pathologies (i) shattering of the chromosome due to asynchrony between the main nucleus and the MN with regards to completion of DNA synthesis and post synthesis condensation of chromatin and the subsequent random ligation of the fragments to generate a hypermutated chromosome and (ii) the pro-inflammatory effect of DNA leakage from MN and NPB with disrupted membranes and shattered chromatin that is sensed by the cGAS-STING (cyclic GMP-AMP Synthase-Stimulator of Interferon Genes) mechanism. The key point here is that MN and NPB formation leads to both chromosome instability and inflammation and explains the association of MN not only with cancer but also other diseases that may be driven primarily by excessive inflammation.

The question of whether DNA damage affects reproductive outcomes (i.e. fertility) and whether it is increased in children exposed to environmental pollutants and children with common diseases is the focus of the review by Knudsen and Kirsch-Volders [6]. Their analysis of the available data indicated a consistent increase in MN frequency of lymphocytes from infertile adults relative to fertile controls, suggesting the possibility that the capacity to produce a sufficient number of functional germ cells may be diminished by genotoxic factors that also increase genomic instability in lymphocytes. Furthermore, MN frequency was also increased in children experiencing common diseases associated with more inflammation, and also in children exposed to genotoxic pollutants in air and water. The mean ratio (MR) values for MN data in lymphocytes and in buccal cells are illustrated in Figs. 1A and 2 respectively. The authors recommended that larger studies are needed to verify these results and also emphasised the practical utility of the minimally invasive buccal cell MN assay when performing studies with children.

Sensing of foreign and self-DNA in the cytoplasm triggers the innate immune system within cells to increase interferon and cytokine production resulting in a pro-inflammatory, autoimmune, and pro-oxidant microenvironment that can increase chromosomal instability. The systematic review and meta-analysis of Kirsch-Volders et al. [7] accumulated and analysed lymphocyte and buccal MN data from studies of diseases in which inflammation and auto-immunity play an important role in their aetiology. The great majority of the studies showed MR values greater than 1.0. Metanalysis of data from diseases with multiple studies found a significant increase in MN in cases compared to controls. The cases to controls ratio of mean MN frequency was higher in buccal cells when compared to lymphocytes suggesting that buccal cell MN frequency may be a more sensitive biomarker of DNA damage in people with excessive inflammation and auto-immune disorders.

Deo et al. [8] focused their review on diseases characterised by elevated levels of advanced glycation end-products (AGEs) and glycated haemoglobin (HbA1c). They reported that lymphocyte and buccal MN frequency was significantly elevated (MR = 1.74, P = 0.0003; MR = 2.86, P = 0.02, respectively) in these diseases. However, there was a lack of significant correlation between the MR for MN and MR for AGEs or HbA1c (measured in the same studies) which suggested that the case-control studies investigated may have been confounded by other variables that affect MN frequency or disease status.

A related review by Franzke et al. [9] specifically examined the

frequency of MN in overweight, obese and diabetic cases in comparison to healthy controls. The studies on overweight and/or obese subjects were insufficient for meta-analysis but there was a tendency for MN in lymphocytes to be elevated in those with body mass index (BMI) values above the normal range. Meta-analysis of lymphocyte MN data from type 1 and type 2 diabetics showed a highly significant increment in MN for the latter ($P < 0.00001$) but only marginal for the former ($P < 0.04$). They also reported on MN data with buccal cells from a subset of 4 studies all of which indicated elevated MN in cases suggesting the potential suitability of this cell type for such studies. Preliminary data on NPB and NBUD in lymphocytes showed similar trends as MN in diabetics.

Chronic kidney disease (CKD) is a growing problem in aging populations world-wide particularly in those with diabetes given that 30–40 % are likely to experience CKD. The aetiology of CKD is complex but it is plausible that either DNA damage predisposes to CKD initiation or progression or that CKD leads to the accumulation of metabolic genotoxins such as homocysteine [10]. Stopper et al. [11] reviewed the papers that have explored the association of MN frequency with CKD in both the predialysis or hemodialysis state of the disease. The results of their meta-analysis showed that DNA damage measured by both the lymphocyte and buccal micronucleus assay is consistently increased relative to healthy controls. They also reported that MN frequency in CKD patients may be modulated by specific medications and micronutrients suggesting that measuring MN in these patients may be useful both as an indicator of disease progression and/or therapeutic efficacy.

Coronary artery disease (CAD) is a leading cause of mortality and there is mounting evidence that DNA damage plays an important role in the development of plaques that cause this affliction [12]. In their review and meta-analysis Andreassi et al. [13] identified eight studies that investigated the association of lymphocyte MN with CAD and with severity of the disease. MN frequency was generally increased in CAD patients ($P = 0.009$) and MN levels were further elevated with higher disease severity ($P = 0.06–0.08$). There was considerable heterogeneity between the studies and for this reason the authors advised that larger and more robust studies are required to control variables that may influence the observed results (e.g type of medication, other complicating pathologies) and to improve the evidence that the MN biomarker can be clinically useful in identifying vulnerable CAD cases.

Fig. 1A illustrates the MR results for all of the above (non-cancer) diseases that had MN frequency data measured in lymphocytes. Fig. 2 illustrates MR data for the above (non-cancer) diseases in which MN in buccal cells were measured. These data are also described in detail in Supplementary Tables 1A and 2.

Four of the papers in the special issue (Bolognesi et al. [14], Asanov et al. [15], Setayesh et al. [16] and Dhillon et al. [17]) focus on MN data of cancer cases relative to controls. These papers covered MN data from studies involving 11 of the most common cancers and also two inflammatory diseases that are associated with increased risk of cancer.

The review of Bolognesi et al. [14] examines the association of MN frequency with head and neck cancers and breast cancer. 81 % of the studies on head and neck cancers measured MN in buccal cells and of these 71 % investigated the association of buccal MN with oral cancer. Buccal MN were significantly increased in oral cancer cases relative to controls (meta-MR = 4.71) and in other head and neck cancer cases (meta-MR = 2.28); similar increments were observed in studies using lymphocyte MN assay. With regards to breast cancer, MN frequency in both lymphocytes and buccal cells was increased in cases relative to controls (meta-MR = 1.90, meta-MR = 3.89, respectively). Family history of breast cancer or carriage of BRCA1/2 mutation did not significantly affect base-line MN or radiation-induced MN in ex vivo lymphocyte cultures. These data suggested that the buccal MN assay

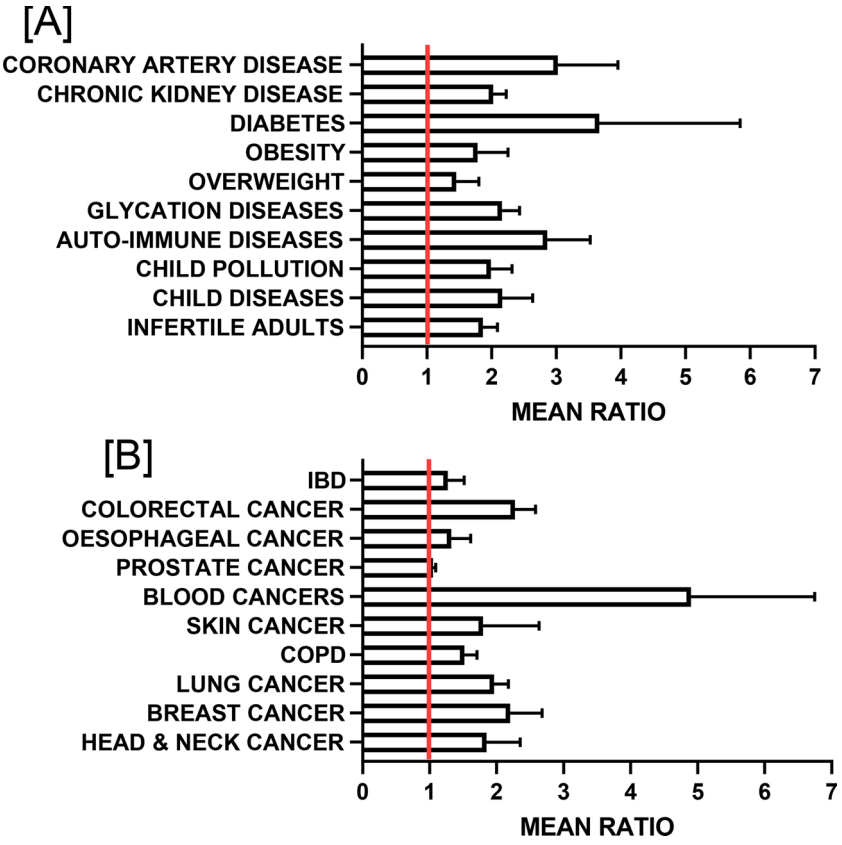


**Fig. 1.** [A] Mean Ratio (MR) of lymphocyte MN values for non-cancer disease cases relative to controls. [B] Mean Ratio (MR) of lymphocyte MN values for cancer and cancer-related disease cases relative to controls. Error bars represent standard error of the mean. IBD, inflammatory bowel disease; COPD, chronic obstructive pulmonary disease.

appears to provide the more promising results which supports further exploration of its clinical utility in identifying subjects with higher risk of head and neck or breast cancer cases.

There is growing interest for discovering biomarkers to identify subjects who may be abnormally susceptible to develop lung cancer. Asanov et al. [15] report the results of a review and meta-analysis on data from 17 studies regarding the relationship between MN frequency and lung cancer; they also explored the relationship of MN with chronic obstructive pulmonary disease (COPD), a condition that predisposes to lung cancer, using results from 4 studies. Fifteen of the 17 studies on lung cancer indicated that lymphocyte MN frequencies that were greater in cases compared to controls (meta-MR = 1.82) and all of the 4 COPD studies had MR > 1 in cases relative to controls (meta-MR = 1.50). Only 3 studies reported results with buccal cells in lung cancer cases and controls but all had MR values >1 (meta-MR = 1.62). The results confirmed the consistency of the association of lymphocyte MN with lung cancer risk but further work is required to consolidate the results with buccal cells which are particularly relevant given that they are located within the aero-digestive tract.

Setayesh et al. [16] focused their review on the diagnostic relevance of MN in urine-derived cells (UDC) and cervix-derived cells (CDC) with regards to cancer of the cervix and of the urothelium (i.e. bladder), respectively. They reviewed 16 relevant studies and found that a strong direct relationship exists between MN frequency and microbial infections, the presence of cancer in the cervix or bladder and the stage of cancer in the cervix. The meta-MR for MN frequency in CDC in cervical carcinoma cases relative to controls, from 18 studies, was calculated to be 8.83 ($p = 0.0000$) in an earlier analysis (Setayesh et al. [18]). Their evaluation of the available data shows that MN are useful additional biomarkers for the detection and prognosis of cervical cancer and possibly also for bladder cancer. However, better standardisation of the UDC and CDC MN assay methodology is essential before translation into clinical practice becomes possible.

In a similar MN and cancer study, Dhillon et al. [17]) analysed the relevant data with regards to the association of lymphocyte MN in skin, haematological, prostate, colorectal and oesophageal cancer cases as compared to healthy controls. Based on data from 19 studies significant increases in lymphocyte MN frequency was observed for subjects with blood cancers (MR = 3.98; $p < 0.0001$) and colorectal cancer (MR = 2.69; $p < 0.0001$) but MR was not significantly increased in prostate, skin and oesophageal cancer. They also investigated inflammatory bowel disease (IBD) because it increases colorectal cancer risk but found no significant association with lymphocyte MN frequency. More studies are needed to further verify and consolidate the degree of associations of lymphocyte MN with the cancers and IBD investigated in this review and meta-analysis.

Fig. 1B illustrates the MR data for all of the above cancers and cancer-related diseases that had MN frequency measured in lymphocytes. Fig. 2 illustrates MR results for the above cancers in which MN in buccal cells were measured, with the exception of the cervical carcinoma and bladder cancer data which were obtained using MN assay in cervix and urothelial derived cells, respectively. These data are also described in detail in Supplementary Tables 1B and 2.

## 3. Comparison of MR values for MN frequency in lymphocytes and exfoliated epithelial cells in cancer and non-cancer diseases

Some notable trends in the MR values for MN frequency and the diseases investigated are as follows:

(i) In all diseases examined, MR values for MN in lymphocytes and exfoliated cells (buccal, cervical, urothelial cell) exceed 1.0 with the exception of prostate cancer.

(ii) The mean (SEM) of MR values for MN in lymphocytes are 2.3 (0.2) and 2.0 (0.3) for non-cancer diseases and cancer respectively. In contrast, the mean (SEM) of MR values for MN in exfoliated cells (buccal, cervical, urothelial cell) are 3.6 (0.4) and 6.1 (1.6) for non-cancer diseases and cancer respectively. The mean MR value for MN in exfoliated cells is significantly greater than in lymphocytes in cancer cases relative to controls ($P = 0.0016$) (Fig. 3A, Supplementary Table 3A).

(iii) The MR values for MN in lymphocytes or exfoliated cells in non-cancer diseases fall within a similar range as the corresponding MR values for cancers and are not statistically different from each other (Fig. 3A, Supplementary Table 3A). This suggests that increase in MN, in the same cell type, is associated with non-cancer diseases to a similar extent as their association with cancer.

(iv) In cancer studies that measured MN in the same tissue in which cancer occurs (e.g., lymphocytes for blood cancer, buccal cells for oral cancer, cervix cells for cervical cells and urothelial cells for bladder cancer) the MR values tended to be higher than those studies in which MN were measured in surrogate tissues (i.e. lymphocytes and buccal cells for studies comparing MN frequencies in cancers that are either not blood or oral cancers respectively). We therefore further stratified the data so that comparisons can be made for cancer-related MR values of MN between the following three groups (a) MR values for MN frequency in lymphocytes for all cancers excluding blood cancers, (b) MR values for MN frequency in exfoliated buccal cells for all cancers excluding oral cancers and (c) MR values for those cases in which MN were measured in cells from the same tissue in which cancer was diagnosed (i.e. lymphocytes for blood cancer, buccal cells for oral cancer, cervix cells for cervical cells and urothelial cells for bladder cancer). This comparison showed that the MR results (mean (SEM)) for the third group (8.4 (1.3)) were much higher as compared to the lymphocyte MN and buccal MN cancer MR results which were 1.7 (0.1) and 2.6 (0.7) respectively with the corresponding statistical significance values being $p < 0.0001$ and $p = 0.0003$ (Fig. 3B, Supplementary Table 3B).

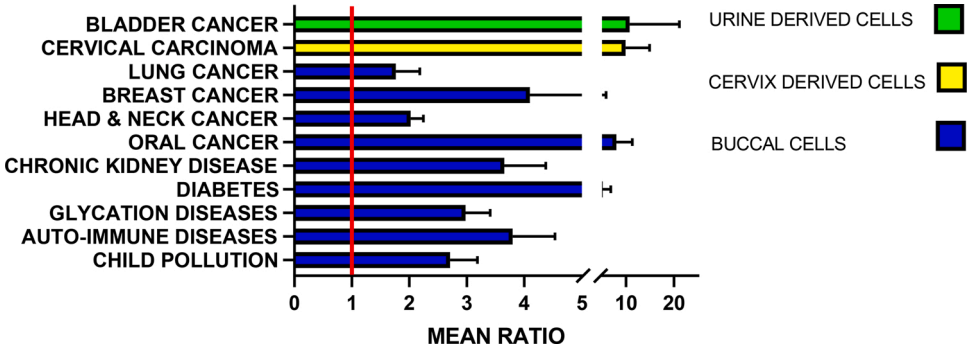


**Fig. 2.** Mean ratio (MR) of exfoliated epithelial cell MN values in cancer or non-cancer cases relative to controls. Error bars are standard error of the mean.

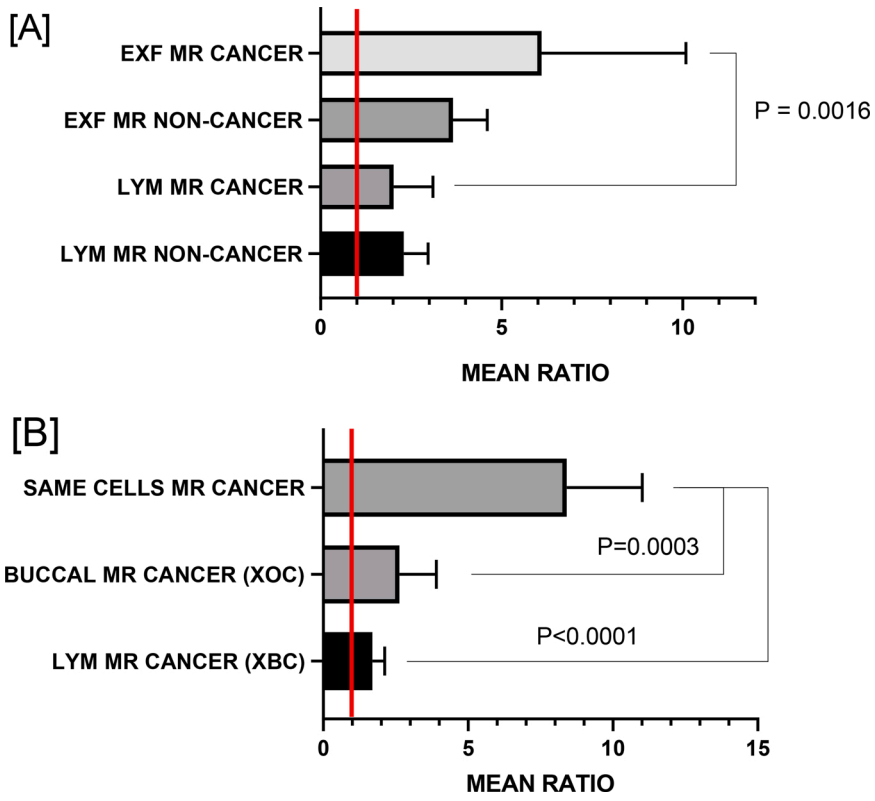


**Fig. 3.** [A] Comparison of mean ratio (MR) values for micronuclei (MN) in lymphocytes (LYM) and exfoliated epithelial (EXF) cells in non-cancer disease and cancer cases relative to controls. LYM MR NON-CANCER = Lymphocyte micronuclei mean ratio in non-cancer disease cases relative to controls. LYM MR CANCER = Lymphocyte micronuclei mean ratio in cancer cases relative to controls. EXF MR NON-CANCER = Exfoliated epithelial cell micronuclei mean ratio in non-cancer disease cases relative to controls. EXF MR CANCER = Exfoliated epithelial cell micronuclei mean ratio in cancer cases relative to controls. [B] Comparison of mean ratio (MN) values in cancer cases relative to controls for (i) MN measured in same tissue as that of the cancer (e.g. lymphocytes for blood cancers, buccal cells for oral cancer, cervix derived cells for cervical cancer, urine derived cells for bladder cancer), (ii) MN measured in buccal cells, in cases with cancer (excluding oral cancer), (iii) MN measured in lymphocytes in cases with cancer (excluding blood cancers). LYM MR CANCER (XBC) = Lymphocyte micronuclei mean ratio in cancer cases (excluding blood cancers) relative to controls. BUCCAL MR CANCER (XOC) = Buccal cell micronuclei mean ratio in cancer cases (excluding oral cancer) relative to controls. SAME CELLS MR CANCER = MR values for cancer cases relative to controls in those cases when MN were measured in cells from the same tissue as that of the cancer. Error bars represent the standard error of the mean.

## 4. The association of MN with ageing and neurodegenerative diseases

Another important aspect of MN assays in lymphocyte and buccal cells is their robust incremental association with chronological ageing and with and accelerated ageing syndromes [19–24] which is driven by mitotic malsegregation of whole chromosomes or chromosome fragments and by the rupture of nucleoplasmic bridges originating from dicentric chromosomes [25]. For these reasons there is considerable interest to explore whether MN, NPB and NBUD may also be prognostic indicators of physical and/or cognitive frailty. In their contribution to this special issue Laffon et al. [26] review the evidence and conclude that "*MN frequency is a suitable biomarker of genomic instability in ageing. However, the possible role of NPB as cytogenetic outcomes indicative of the ageing process is still unclear and further investigation is required to figure this out.*". Furthermore, they discuss the limited evidence from two studies exploring the association of MN with frailty and the limitations of the study designs that need to be addressed in further studies in this emerging field.

There is also much interest in the association of MN with neurodegenerative diseases which were originally initiated by the work from Lucia Migliore's group showing significant increments in MN frequency in Alzheimer's and Parkinson's disease cases that were shown to be due to chromosome loss in the former and chromosome breakage in the latter [24,27]. Further studies by Fenech's group showed that MN were only marginally increased in buccal cells but not in lymphocytes, and that in lymphocytes MN, NPB and NBUD were inversely correlated with cognitive function measured in healthy controls, mild cognitive impairment cases and Alzheimer's disease cases [24,28]. In a more recent and larger study of various neurodegenerative disorders Reimann et al. [29] could not find significant associations of buccal MN and other related biomarkers (e.g. nuclear buds, binucleated cells) with the disease state when compared to controls. These contrasting relationships between these chromosome instability biomarkers and neurodegenerative diseases highlight the need for better study designs to obtain more robust data that can be replicated across laboratories. Large heterogeneity and paucity of studies since the previous review on this topic [24] hindered the possibility of a meta-analytical approach to address the question of the association of MN and other nuclear anomalies with neurodegenerative diseases such as Alzheimer's disease. Consequently, the review on neurodegenerative diseases and MN has been delayed until such time when sufficient consistent comparable data is available and for this reason it is not included in this special issue.

Given the importance of genomic instability in the aetiology of age-related disease, one of the reviews of the special issue was dedicated to studies that explored the possibility of reducing the level of MN, NPB and NBUD by diet and/or lifestyle interventions in the elderly. The systematic review of Wagner et al. [30] identified 13 papers describing dietary and/or physical activity interventions in the elderly that measured MN. Nine of these studies were deemed suitable for meta-analysis because they involved a randomised controlled trial (RCT), in those 60 years of age or older and the intervention was directed towards reducing risk of common age-related diseases or micronutrient deficiency and included MN and/or NPB or NBUD in lymphocytes or buccal cells as biomarkers of effect. Apart from the tea intervention by Li N et al. [31] no other RCT identified in the meta-analysis significantly reduced MN in lymphocytes. The same intervention [31] also reported significant reductions in MN in buccal cells, and so did the antioxidant supplementation studies of Stich et al. [32,33]. The lack of effect in the other interventions might have been due to limitations of study design such as lack of participant screening which is necessary to identify likely responders based on their deficiency in the nutrients used in the intervention and, also, on the basis of their elevated frequency in MN, because it is generally those who are micronutrient deficient and/or exhibit elevated base-line levels of MN who respond to intervention. The efficacy of this approach was for example demonstrated in a RCT intervention with folic acid and vitamin B-12 supplementation in young adults (Fenech et al. [34]) and an RCT of zinc carnosine supplementation in apparently healthy elderly subjects with low zinc status (Sharif et al. [35]). It is evident that stricter

attention to study design and participant selection is required to obtain robust scientific evidence that proves the efficacy of interventions to improve genome integrity in both younger and older adults.

## 5. Knowledge gaps and future research required to translate MN assays into clinical practice

The final paper of the special issue by Bonassi and Fenech [36] discusses a roadmap of what is required to validate and translate the application of MN assays into clinical practice with the support of health care professionals. The proposed roadmap consists of three critical steps:

(i) Evidence that MN assays can differentiate disease subjects vs unaffected individuals at group level using Case-Control studies. These studies should also identify important variables such as demographic, genetic, environmental, therapeutic and nutrition factors that can modify the MN biomarker in healthy and disease subjects.
(ii) Transition of the use of MN assays from application at the group level to the individual level. This requires technological advances to establish minimally invasive high-throughput MN measurement techniques so that MN assay data at the individual level are statistically sufficiently robust to be adequately reliable and affordable to be deployed in a clinical setting.
(iii) Prospective cohort studies and randomised controlled trials to verify that MN assays are predictive of disease and that MN frequency modification alters disease outcomes.

After the realisation of these three critical steps, pragmatic trials will also be required to evaluate the usefulness of MN measurements in routine clinical practice to provide the decisive evidence to support their adoption by the medical and public health community. The review also provides examples of MN assays that are currently in clinical practice and others for which there is already adequate evidence for considering pragmatic trials for their translation into practice.

A strength of the MR data generated from papers in the SI is the *a priori* definition of common guidelines for the selection of papers, including search strategy, acceptability of scoring criteria and availability of a relevant control population. A potential weakness of the MR data generated in the reviews is that most of the reviewed studies used only one cell type to determine the association of MN with a particular disease. Consequently, comparisons of MN association with disease, using MN data from different cell types, involved data that often was not from the same individual, which may have increased variability in the MN data. Therefore, more studies are needed that also compare MN frequency in different tissues from the same individuals to determine more robustly the degree of correlation of MN frequency between different tissues and, to establish, more reliably, for each tissue the degree of association of MN with important diseases. The relative sensitivity of the association of MN frequency with specific diseases probably depends on whether the cells sampled are from a tissue that is directly or indirectly exposed to exogenous or endogenous genotoxins that contribute to the aetiology of the disease. Fig. 4 illustrates the inter-relationship between endogenous/exogenous genotoxin, glycation and inflammation exposure and the potential direct or indirect exposure routes of tissues for which MN assays have been established.

Up to now five prospective studies have been reported showing that elevated lymphocyte MN frequency predicts cancer risk, cardiovascular disease mortality, adverse cardiac events and pregnancy complications [37–41]. More prospective studies are needed to consolidate these observations and also to test whether similar results are obtained using MN data from buccal cells and possibly other cell types from the tissue where the disease occurs. Ideally, as a minimum, future prospective studies should include data for both lymphocyte and buccal MN frequency from the same individual.

## 6. Conclusions

This special issue is an important milestone in the evidence supporting the application of MN as a plausible genomic biomarker of developmental and degenerative disease risk. There is now an abundance of evidence from case-control studies that MN in lymphocytes measured using the cytokinesis-block MN assay in lymphocytes and conventional MN assay in buccal cells is substantially elevated in both non-cancer diseases and in the great majority of cancers. Based on the MR data evaluated in this SI, the association of MN in buccal cells with disease appears to be as robust as MN in lymphocytes. Furthermore, measuring MN in the tissue in which cancer arises is more strongly

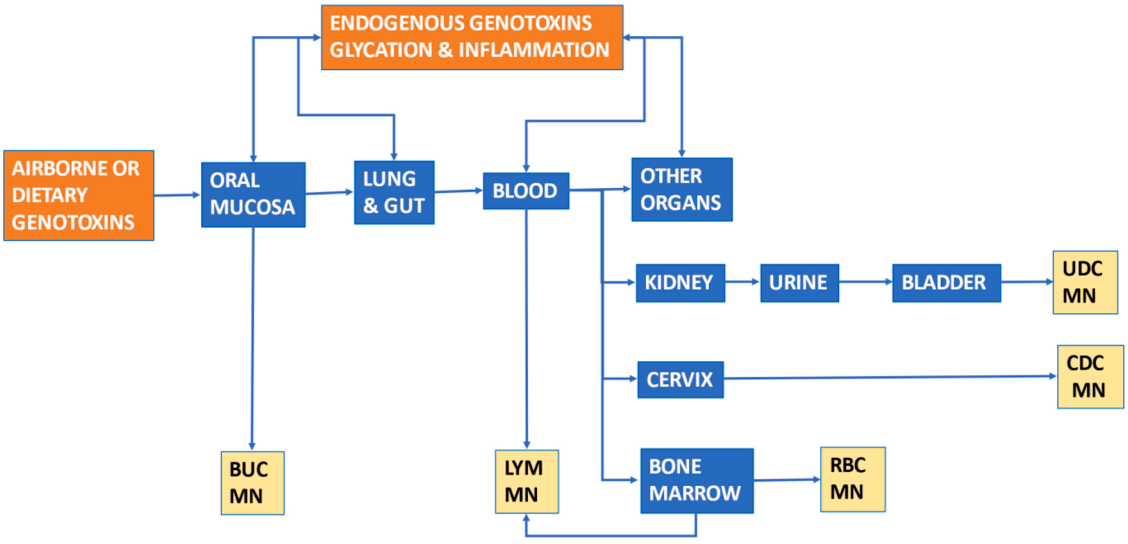


**Fig. 4.** Flow diagram of inter-relationship between endogenous/exogenous genotoxin, glycation and inflammation exposure and their sequential impact on micronucleus (MN) formation in tissues for which MN assays have been established (BUC MN, micronuclei in buccal cells; CDC MN, micronuclei in cervix derived cells; LYMN MN, micronuclei in lymphocytes; RBC MN, red blood cell micronuclei; UDC MN, micronuclei in urine derived cells). Other factors not shown in the diagram that influence MN formation include genetic susceptibility to genotoxin-induced micronuclei and the aggravating effect of deficiency in certain micronutrients (e.g. folate, vitamin B-12 and zinc deficiency) that are required for DNA replication and repair and are known to increase MN formation when their supply is inadequate.

associated with cancer risk than measuring MN in surrogate tissue, however, this is an approach that may only be practical in the clinical setting depending on the location and accessibility of the cancer being investigated. These important advances underscore the need to identify the diseases where current evidence suggests the best chance of success in translating MN assays into practice and to develop appropriate study designs to achieve these purposes.

## Declaration of Competing Interest

The authors report no declarations of interest.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mrrev.2021.108384.

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