[Downloaded free from http://www.ejcrp.org on Tuesday, September 20, 2022, IP: 252.195.115.106]

Journal of Cancer Research and Practice

journal homepage: www.ejcrp.org

Original Article

# Significance of Morphological Markers of Chromosomal Instability in Differentiating Malignant and Benign Effusions: A Pilot Study in Rural Central India

**Abhay Vilas Deshmukh[1], Akshay Sudhir Yadav[2], Vitaladevuni Balasubramanyam Shivkumar[1*]**

[1]Department of Pathology, Mahatma Gandhi Institute of Medical Sciences, Wardha, Maharashtra, India
[2]Department of Anaesthesiology, Lokmanya Tilak Municipal Medical College, Mumbai, Maharashtra, India

## Abstract

**Background:** Chromosomal instability (CI) is critical for carcinogenesis. The morphological markers of CI include multipolar mitosis (MPM), chromatin bridge (CB), micronuclei (MN), and nuclear bud (NB). These represent an underlying genetic instability and can be studied in routine cytological specimens. The aim of this study was to evaluate the significance of morphological markers of CI in differentiating malignant and benign effusion smears. **Materials and Methods:** In this retrospective observational pilot study, 25 cases of benign and 25 cases of malignant effusion smears were selected. All of the malignant cases were reconfirmed by histopathology for primary sites. One thousand cells in May–Grunwald–Giemsa-stained smears were counted for MPM, CB, MN, and NB. The significance of these markers of CI was compared between the benign and malignant cases. **Results:** The mean numbers of MPM, CB, MN, and NB in malignant cases were 10.52, 7.72, 1.36, and 0.40 per 1000 cells counted, compared to 0.7, 0.5, 0.3, and 0 per 1000 cells counted in benign cases, respectively. The Student's *t*-test showed highly significant differences between the benign and malignant effusion smears for the CI markers, with $P < 0.000001$, $< 0.000001$, and $<0.00001$ for MN, NB, and MPM, respectively. **Conclusion:** There were significant differences in the scores of morphological markers of CI in cytological smears between malignant and benign effusions. This is a convenient and reliable method to differentiate between malignant and benign effusions and can be used in conjunction with cytomorphology if a larger study is able to establish the significance in effusions.

**Keywords:** Carcinogenesis, chromosomal instability, effusion, morphological markers

## Introduction

The diploid chromosome number in a cell is maintained due to faultless mitotic segregation. Chromosomal instability (CI) refers to an unequal distribution of DNA to

**Address for correspondence:**
Dr. Vitaladevuni Balasubramanyam Shivkumar,
Department of Pathology, Mahatma Gandhi Institute of Medical Sciences,
Sevagram, Wardha - 442 102, Maharashtra, India.
E-mail: shivkumar@mgims.ac.in

**Submitted:** 15-Dec-2021 **Revised:** 08-Mar-2022
**Accepted:** 10-Mar-2022 **Published:** 02-Sep-2022

Access this article online

Quick Response Code:

Website: www.ejcrp.org

DOI: 10.4103/JCRP.JCRP_3_22

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

**For reprints contact:** WKHLRPMedknow_reprints@wolterskluwer.com

**How to cite this article:** Deshmukh AV, Yadav AS, Shivkumar VB. Significance of morphological markers of chromosomal instability in differentiating malignant and benign effusions: A pilot study in rural central India. J Cancer Res Pract 2022;9:97-100.

[Downloaded free from http://www.ejcrp.org on Tuesday, September 20, 2022, IP: 252.195.115.106]



the daughter cells during mitosis and is a critical event in carcinogenesis.[1-3]

Multipolar mitosis (MPM), chromatin bridge (CB), micronuclei (MN), and nuclear bud (NB) are known indicators of CI.[4-6] Different sophisticated techniques such as flow cytometry, fluorescence *in situ* hybridization, DNA ploidy, AgNOR staining, immunostaining with specific antibodies against centromere and telomere, immunohistochemistry, and fluorescence and time-lapse microscopy have been used to study CI.[5] Morphological markers of CI represent an underlying genetic instability, and they can be studied in routine cytological effusion specimens to differentiate between benign and malignant lesions.[6]

The aim of the present study was to analyze the significance of morphological markers of CI, namely MPM, CB, MN, and NB in effusions, and their significance in distinguishing between benign and malignant effusions. This project was conducted as a pilot study before carrying out a study with a large sample size.

## Materials and Methods

This retrospective observational pilot study was approved by the Ethics Committee of Mahatma Gandhi Institute of Medical Sciences (MGIMS), Sevagram, Wardha, Maharashtra, India (vide approval letter number MGIMS/IEC/235/2015, dated September 8, 2015). It was conducted at the cytopathology section of the Department of Pathology at MGIMS, a rural tertiary care teaching institute in central India over a period of 1 year (from December 2018 to November 2019). Informed consent was obtained from the patients/relatives before performing ascitic and pleural fluid tap. Patient confidentiality was maintained throughout the research procedure.

### Patient selection

We selected slides of 25 cases of benign and 25 cases of malignant effusions from archives of the department. One May–Grunwald–Giemsa (MGG)-stained cellular smear for each case was examined to study CI markers. Each smear was screened for MPM, CB, MN, and NB per 1000 cells by two independent observers.

In Giemsa-stained smears, the diameter of MN varied from 1/16 to 1/3 of the diameter of the main nucleus, and the color and texture were similar or slightly darker to the main nucleus [Figure 1a].[6] CBs were identified as perpendicularly aligned amphophilic stained connecting filament lying between two well-separated parallel anaphase plates [Figure 1b].[4] NBs were identified as structures resembling miniature nuclei seen as sessile or pedunculated bud-like protrusions from the nucleus. NBs contain interstitial or terminal acentric fragments more commonly than the whole chromosome and are attached to the nuclear membrane by a narrow stalk [Figure 1c].[7] Chromosomal segregation in MPM takes place in a multipolar fashion and results in unequal distribution of chromosomes to daughter cells [Figure 1d].[6]

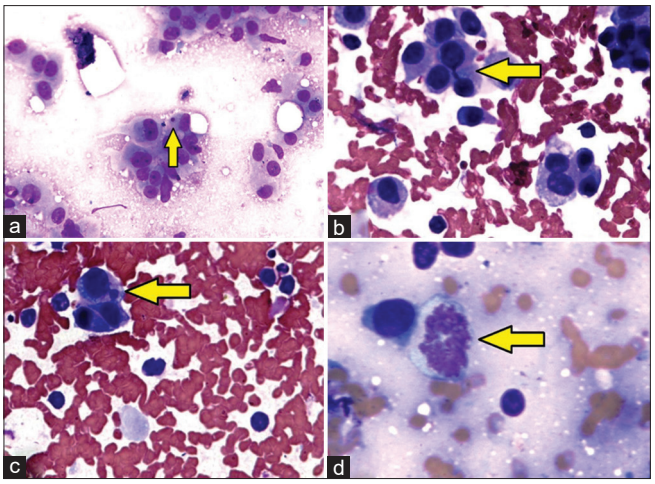


**Figure 1:** Smear showing morphological markers of chromosomal instability; (a) Micronucleus (arrow) in a case of metastatic adenocarcinoma cells in ascitic fluid (Giemsa, ×400), (b) Chromatin bridge (arrow) in a case of metastatic adenocarcinoma cells in pleural fluid (Giemsa, ×400), (c) Nuclear bud (arrow) in a case of metastatic adenocarcinoma cells in pelvic washing (Giemsa, ×400), (d) Multipolar mitosis (arrow) in a case of metastatic adenocarcinoma cells in pleural fluid (Giemsa, ×1000)

Almost 30 min was required for scoring in each case. The significance of each morphological marker of CI was analyzed between benign and malignant cases. The mean numbers of MN, NB, CB, and MPM in malignant and benign effusions were calculated.

### Inclusion criteria

All of the malignant effusion cases were those with a confirmed diagnosis of adenocarcinoma from different primary sites on histopathology in a cell block study, pleural and/or peritoneal biopsy. Benign effusion cases were negative for malignant cells on cytology and on clinical follow-up. Cases of reactive mesothelial hyperplasia on cytology were selected as benign cases.

### Exclusion criteria

Smears with inadequate cellularity (<2000 cells/smear), severely obscured background due to dense inflammation, necrosis, and other artifactual changes and cases with a history of previous chemotherapy or radiotherapy were excluded from the study.

### Statistical analysis

Statistical analysis was done using descriptive and inferential statistics using the Student's *t*-test, mean, and standard deviation (SD). SPSS version 17.0 (IBM Corp. Released 2011. IBM Statistics for Windows, (Armonk, New York, United States) Version 20.0. (Armonk, New York, United States) and GraphPad PRISM version 5.0 (GraphPad Prism, Version 5.0: San Diego, California, USA) were used for all analyses, and $P < 0.05$ was considered to be a minimum level of significance.

## Results

The study included 50 effusion smears from different sites, including pleural fluid ($n = 31$), ascitic fluid ($n = 13$), peritoneal

[Downloaded free from http://www.ejcrp.org on Tuesday, September 20, 2022, IP: 252.195.115.106]



washing ($n = 4$), and pelvic washing ($n = 2$). Twenty-five cases were benign and 25 cases were malignant. The primaries in the malignant effusion smears are shown in Table 1.

### Morphological markers

The mean MN score was 10.52/1000 cells in malignant smears and 0.70/1000 cells in benign smears (SD 3.50 and 0.87, respectively). The mean NB score was 7.72/1000 cells in malignant smears and 0.50/1000 cells in benign smears (SD 3.13 and 0.58, respectively). The differences in scores between the malignant and benign effusions were statistically highly significant ($P < 0.000001$ for MN and NB). The mean MPM score was 1.36 in malignant smears and 0.30 in benign smears (SD 0.95 and 0.47, respectively). The difference in score between the malignant and benign effusion was statistically highly significant ($P < 0.00001$). The mean CB score in malignant smears was 0.40 (SD 0.58); however, it was absent in benign smears [Table 2].

## Discussion

In effusion cytology, the distinction between benign and malignant effusion is very critical from the treatment point of view. In most cases, a clear distinction between benign effusion and malignant effusion can be made on cytomorphology alone; however, there are a few cases in which this distinction is challenging, mainly due to overlap in cytomorphological features of reactive mesothelial cells and adenocarcinoma cells. In these cases, apart from other ancillary techniques such as immunohistochemistry, morphological markers of CI may have a role to play in differentiating between benign and malignant effusions. CI plays an important role in carcinogenesis. The term CI denotes unequal distribution of genomic material during mitosis, leading to changes in chromosomal structure and number, increased segregation errors during cell division, and mutations, all of which promote carcinogenesis.[8]

Advanced techniques can be used to detect chromosomal abnormalities in malignancies, including the use of genomic hybridization and fluorescence *in situ* hybridization (FISH) techniques.[9] These techniques have high sensitivity of detection but are expensive, time-consuming, tedious, and need specifically trained personnel. In resource-constrained settings, the routine use of these techniques is not feasible. Morphological markers of CI, namely MN, CB, MPM, and nuclear budding (NB) can easily be measured from routine cytology smears with the help of a light microscope.

The morphological markers of CI are formed during cell division. MN is a small additional nucleus lying within the cytoplasm. Its size is approximately one-third of the nucleus. It is formed whenever a chromosome or its fragment is not incorporated into one of the daughter nuclei during cell division, and it is considered to be a sensitive marker of CI.[6] CB is formed during anaphase. In this phase, centromeres of dicentric chromosomes are pulled to opposite poles during mitosis. CB is nothing but a perpendicularly aligned amphophilic connecting filament between two separate parallel plates.[4] Chromosomal separation in a multipolar fashion results in the unequal distribution of chromosomes to daughter cells, leading to the formation of MPM.[6] NBs are similar to miniature nuclei and are sessile or pedunculated bud-like protrusions which contain terminal or interstitial acentric fragments are attached to the nuclear membrane by a narrow stalk.[7]

Studies have shown an increase in the number of these morphological markers of CI in malignancies of buccal mucosa,[10] cervical smears,[11] urothelial cells,[12] breast lesions,[4,13] and pancreatic lesions[14] compared to their benign counterparts. CI is related to poor survival in carcinoma patients.[8] Studies have also shown that CI is related to an increased risk of carcinoma, and thus, it can be used in cancer surveillance.[15] If properly utilized, these morphological

**Table 1: Total number of malignant effusion cases according to the primary sites**

| Sites | Number of cases |
|---|---|
| Breast | 7 |
| Lung | 5 |
| Ovary | 5 |
| Colon | 4 |
| Stomach | 2 |
| Gallbladder | 1 |
| Pancreas | 1 |
| Total | 25 |

**Table 2: Mean values of various morphological markers of chromosomal instability (micronucleus, nuclear budding, multipolar mitosis, and chromatin bridge) in benign and malignant cases**

| | Micronucleus | Nuclear budding | Multipolar mitosis | Chromatin bridges |
|---|---|---|---|---|
| Mean | | | | |
| Malignant ($n$=25) | 10.52 | 7.72 | 1.36 | 0.40 |
| Benign ($n$=25) | 0.70 | 0.50 | 0.30 | 0 |
| SD | | | | |
| Malignant ($n$=25) | 3.50 | 3.13 | 0.95 | 0.58 |
| Benign ($n$=25) | 0.87 | 0.58 | 0.47 | 0 |
| $P$ | <0.000001 | <0.000001 | <0.00001 | NA** |
| $t$* | 14.12 | 11.7 | 5.15 | NA** |

*Student's $t$-test. NA: Not applicable, SD: Standard deviation

[Downloaded free from http://www.ejcrp.org on Tuesday, September 20, 2022, IP: 252.195.115.106]



markers of CI can be used to identify high-risk patients as well as for prognosis in certain cancer patients.[6] One of the disadvantages of these morphological markers is that it takes around 30 min for scoring, and the strict criterion of counting 1000 well-preserved cells has to be followed. Our study showed that it was easy to measure MN and NB in cytology smears as they occurred with higher frequency. Identification of CB and MPM was harder due to the need for more extensive searching and the presence of crushing artifacts and nuclear debris.

Very few studies have shown a strong correlation between markers of CI and cytological diagnosis of malignancy in effusions.[6] Kaur and Dey[16] evaluated the role of scoring MN to distinguish between benign reactive mesothelial cells and adenocarcinoma cells in effusion fluids. We studied four morphological markers of CI (CB, MPM, MN, and NB) in cytology smears with MGG staining. The results of our study showed that the occurrence of these markers of CI was very common in the malignant effusions compared to the benign effusions, and the differences were statistically significant (MN, $P < 0.000001$; NB, $P < 0.000001$; and MPM, $P < 0.00001$).

In this study, we did not include cases with atypical or suspicious cells in effusion specimens. We did not use immunostaining to confirm the malignancy cases. In addition, we did not use different sophisticated and advanced techniques such as genomic hybridization, DNA ploidy, FISH, AgNOR staining, immunostaining with specific antibodies against centromere and telomere, immunohistochemistry, and fluorescence and time-lapse microscopy for CI. These are possible limitations of this study. However, this study was mainly conducted to explore the possibility of replacing these sophisticated, expensive techniques to estimate chromosomal abnormalities with morphological markers, especially in resource-constrained centers. Only a few studies have established the significance of morphological markers of CI in differentiating malignant and benign effusions to date.[6,16] To the best of our knowledge, this is the first pilot study in central India on this issue. Thus, we suggest that a larger study with validation and test cases (including suspicious/atypical) along with comparisons with immunostaining (cells blocks) is warranted to clarify the exact roles of morphological markers of CI in differentiating malignant and benign effusions.

## Conclusion

This study reports a simple technique to identify and count markers of CI (CB, MN, MPM, and NB) by examining routine MGG-stained cytology smears with the use of light microscopy. Evaluating these markers in routine reporting may be useful to differentiate malignant effusions and benign effusions, especially where the distinction is difficult on cytomorphology alone if a larger study is able to establish the significance in effusions.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

## References

1. Weaver BA, Cleveland DW. Does aneuploidy cause cancer? Curr Opin Cell Biol 2006;18:658-67.
2. Boveri T. Concerning the origin of malignant tumours by Theodor Boveri. Translated and annotated by Henry Harris. J Cell Sci 2008;121 Suppl 1:1-84.
3. Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. Nature 1998;396:643-9.
4. Verma S, Dey P. Correlation of morphological markers of chromosomal instability in fine needle aspiration cytology with grade of breast cancer. Cytopathology 2014;25:259-63.
5. Camps J, Ponsa I, Ribas M, Prat E, Egozcue J, Peinado MA, *et al.* Comprehensive measurement of chromosomal instability in cancer cells: Combination of fluorescence *in situ* hybridization and cytokinesis-block micronucleus assay. FASEB J 2005;19:828-30.
6. Tyagi R, Dey P, Uppal R, Rajwanshi A. Analysis of morphological markers of chromosomal instability in ascitic fluid. Diagn Cytopathol 2015;43:855-8.
7. Pampalona J, Roscioli E, Silkworth WT, Bowden B, Genescà A, Tusell L, *et al.* Chromosome bridges maintain kinetochore-microtubule attachment throughout mitosis and rarely break during anaphase. PLoS One 2016;11:e0147420.
8. Jin Y, Stewénius Y, Lindgren D, Frigyesi A, Calcagnile O, Jonson T, *et al.* Distinct mitotic segregation errors mediate chromosomal instability in aggressive urothelial cancers. Clin Cancer Res 2007;13:1703-12.
9. McGranahan N, Burrell RA, Endesfelder D, Novelli MR, Swanton C. Cancer chromosomal instability: Therapeutic and diagnostic challenges. EMBO Rep 2012;13:528-38.
10. Halder A, Chakraborty T, Mandal K, Gure PK, Das S, Raychowdhury R. Comparative study of exfoliated oral mucosal cell micronuclei frequency in normal, precancerous and malignant epithelium. Int J Hum Genet 2004;4:257-60.
11. Samanta S, Dey P, Nijhawan R. Micronucleus in cervical intraepithelial lesions and carcinoma. Acta Cytol 2011;55:42-7.
12. Arora SK, Dey P, Saikia UN. Micronucleus in atypical urothelial cells. Diagn Cytopathol 2010;38:811-3.
13. Hemalatha A, Suresh TN, HarendraKumar ML. Micronuclei in breast aspirates. Is scoring them helpful? J Cancer Res Ther 2014;10:309-11.
14. Matsuda Y, Yoshimura H, Ishiwata T, Sumiyoshi H, Matsushita A, Nakamura Y, *et al.* Mitotic index and multipolar mitosis in routine histologic sections as prognostic markers of pancreatic cancers: A clinicopathological study. Pancreatology 2016;16:127-32.
15. El-Zein RA, Schabath MB, Etzel CJ, Lopez MS, Franklin JD, Spitz MR. Cytokinesis-blocked micronucleus assay as a novel biomarker for lung cancer risk. Cancer Res 2006;66:6449-56.
16. Kaur J, Dey P. Micronucleus to distinguish adenocarcinoma from reactive mesothelial cell in effusion fluid. Diagn Cytopathol 2010;38:177-9.