Validating Micronucleus Score in Effusion Fluids

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Abstract

Background: Identifying malignant cells in effusion fluid is vital in staging and management of cancers. Differentiating reactive mesothelial cells from malignant cells in effusion fluid is a challenging task and there is an ongoing need for simpler and cost effective tool to aid the diagnosis. Micronucleus is an additional smaller nucleus in the cytoplasm, formed by chromosomes or chromosomal fragments formed during cell division. Aims: The aim of this study was to assess the significance of micronucleated cell in effusion fluids to distinguish adenocarcinomatous from reactive mesothelial effusions. Materials and Methods: Thirty cases of unequivocal malignant effusion fluids and 30 benign cases with reactive mesothelial cells as control were studied. Number of microucleated cells present per1000 well-preserved cells in Leishman-stained smears were counted. Results: Mean (±SD) micronucleated score in malignant and benign effusions were 15.77 ± 9.78 and 1.87 ± 1.78, respectively. The median scores were 13 and 2, respectively. Mann–Whitney test showed that this difference was statistically significant (P < 0.001). This study revealed that there was a significant difference in micronucleus scoring between benign and malignant effusions. Conclusions: Micronucleus score can be used as an additional biomarker in the interpretation of routinely stained cytosmears.

Keywords: Adenocarcinoma, effusion fluid, micronucleus

INTRODUCTION

Identifying malignant cells in effusion fluid is of paramount importance in staging and management of cancers. Differentiating reactive from malignant cases in effusion fluid is difficult at many times. We need a simple, inexpensive, and reliable tool to identify them. Micronucleus (MN) test is a simple test which is done on routine staining, thus avoiding the need of expensive methods and the use of immunocytochemistry panels and flow cytometry.[1–4]

MN is an additional smaller nucleus in the cell cytoplasm. Its presence indicates chromosomal damage or breakage and mitotic dysfunction of the cell. MN is round to oval in shape and is separated from the main nucleus. The mean diameter is 1/16th to 1/3rd of the main nucleus and is nonrefractile. Color and texture is the same as that of the main nucleus.[6,7]

Utility of MN scoring have been studied in cervical smears,[8,9] exfoliated buccal and bladder cells,[10] colorectal carcinoma,[11] and lymphocytes[12] to detect chromosomal damage, exposure to carcinogens, and genotoxic agents.

Because limited number of MN studies has been reported on effusion fluids, the present study was undertaken to distinguish malignant cells from reactive mesothelial cells.

The aim of this study was to assess the significance of micronucleated cells in effusion fluids to distinguish malignant cells from reactive mesothelial cells in unequivocal cases. Herein, we found out the utility of micronucleated cell scoring in cytological smears of effusion fluid to distinguish benign and malignant effusions.

MATERIALS AND METHODS

The present study was carried out in our department after obtaining approval from the ethical research committee. A total of 60 cases sent for fluid analysis were considered for the study, 30 were malignant and 30 were benign (reactive).

Relevant clinical details including the age, sex, type of effusion, and site of malignancy were noted from the departmental data base. Additional information regarding radiological findings/tumor markers was collected from hospital records.

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All malignant cases included in the study were supported either by histopathology or by radiology. Benign cases included were approved by three cytopathologists independently as benign.

Smears of diagnosed cases of metastatic adenocarcinoma and reactive mesothelial cells stained with Leishman stain were studied for MN scoring. The smears were analyzed by light microscopy under oil immersion (1000×) separately and independently by two scorers and an average score was calculated. Only the noninflammatory cells were considered for scoring. Separately lying cells without overlap were studied. The number of micronucleated cells per 1000 well-preserved cells under oil immersion lens (1000×) was counted. MN were detected according to the following criteria:

1. Should be within the cytoplasm of the cell
2. Round to oval in shape and nonrefractile
3. Mean diameter less than 1/3 of the main nucleus
4. The shape, color, and texture of MN are similar to those of the original nuclei
5. It should be in the same optical plane as the original nucleus
6. Distinctly separate from the main nucleus with a similar staining intensity.

Degenerated cells, apoptotic cells, cytoplasmic fragments, and overlapping and inflammatory cells were exempted from counting and scoring. Cells with single/multiple MN were counted as one.

Descriptive statistics used was mean (SD) for continuous variables and percentage for categorical and dichotomous variables. Receiver operator characteristic (ROC) analysis was used to determine the sensitivity and specificity. Mann–Whitney test (nonparametric test) was used to detect MN score difference between two groups. MN score in both the groups was expressed as mean ± SD and median (min–max) where appropriate. Area under the curve (AUC) is a measure of the overall performance of a diagnostic test. It was assessed using ROC curve analysis to determine the cut-off point between the two groups. The ROC plots the true positive rate (sensitivity) against the false-positive rate (1-specificity). The closer the AUC is to 1 the better is the overall diagnostic performance of the test, and a test with an AUC value of 1 is one that is perfectly accurate. The practical lower limit for the AUC of a diagnostic test is 0.5. Data processing and analysis were performed using SPSS Statistics for Windows (version 20.0. Armonk, New York: IBM corporation).

**Results**

The present retrospective study was done on 60 effusion fluids which included 30 benign (reactive) and 30 malignant cases. Age ranged 33–85 years and females had a higher preponderance than males. The mean ± SD number of MN score in malignant cases was 15 ± 9.78 with a maximum of 50 and a minimum of 6. Median score was 13. Maximum number of MN seen in a cell was 3. The mean ± SD number of MN score in benign cases was 1 ± 1.78 with a maximum of 5 and minimum of 0. Median score was 2 [Table 1]. Mann–Whitney test scoring revealed a statistically significant difference in MN score between the two groups. The results of the ROC analysis showed that MN counts possessed a high degree of sensitivity and specificity. Using the ROC curve method, we found out a cut-off of 5.5 for MN count giving a sensitivity of 100% and a specificity of 100% at this point ($P = 0.000$ and AUC = 1).

**Discussion**

To the best of our knowledge there are currently only two published studies of MN scoring on effusion fluids, however, these studies have been done on other samples such as exfoliated buccal, vaginal, cervical, and lymphocytes. These studies noticed significant difference in MN scoring between benign and malignant groups. The present study was undertaken to examine the difference in MN scoring between the two groups (benign and malignant) objectively, and if possible, to evaluate the cut-off point between the two groups by validation of MN score in effusion fluids.

The study conducted by Kaur and Dey on ascitic fluid effusions using MGG stain included 20 and 15 cases of malignant cells and benign mesothelial cells, respectively.[14] In their retrospective study, test group age ranged from 34 to 71 years and females were more than males. They concluded that MN scoring was significantly higher in malignant effusions and their mean MN score in malignant cases was 21 compared to 2.9 in benign cases. Another study done by Tyagi et al. on ascitic fluid including 60 benign and 40 malignant fluids showed mean MN score in malignant group to be 13.2 and

| Category | Number of cases | MN score mean±SD (min-max) | Median |
|----------|----------------|---------------------------|--------|
| Malignant| 30             | 15.77±9.78 (6-50)         | 13     |
| Benign   | 30             | 1.87±1.78 (0-5)           | 2      |
the MN score ranged from 1 to 58. Benign group showed mean a MN score of 0.57 ranging from 0 to 5.15 Our results correlate with their results [Table 2]. The standard deviation showed wide variation in malignant group and were 9.78 and 1.78 in malignant and benign groups, respectively. A similar finding was noted in many other studies. They suggested that more variation in malignant cases was probably due to type and staging of the primary tumor, carcinogen exposure, and genetic causes. We considered few additional factors to explain this variation in fluid cytology. First factor may be the variable admixture of reactive mesothelial cells with malignant cells in malignant effusions which might lead to extreme differences in MN scoring. It is well-known that malignant effusions also show reactive mesothelial cells. Admixture of more reactive mesothelial cells might have led to low scores. Second, the presence of malignant signet ring cells pose another problem as these cells have peripherally compressed cytoplasm which may obscure the detection of micronucleus, leading to a low score [Figure 1 inset]. These problems were unique to effusions and not noticed in vaginal or oral smears.

MN scoring can be difficult in situations, as stated in Table 3, where structures mimicking MN such as stain deposits, nuclear debris, overlapping platelets, and lymphocytes were present [Figure 2]. When encountered with such problems, comparing the structures with texture and staining characteristics of original nucleus helps us to isolate the artefacts. Apoptotic cells also mimic MN and can be easily differentiated by noting the absence of original (main) nucleus. The problems of keratohyaline granules mimicking MN were seen only in studies where squamous cells were seen. The present study included only adenocarcinoma and we did not encounter this problem. We did not find any significant difference in MN scoring in relation to age and site of primary cancer. Normal cells during cell division can also rarely give rise to micronucleus, however, this is a less common phenomenon when compared to preneoplastic and neoplastic conditions.

MN scoring is a simple and effective tool to distinguish between benign and malignant effusion in routine cytological analysis. The limitations of our study were smaller sample size and absence of use of specific nuclear stains. Hence, we advocate prospective larger scale studies with adequate sample size and use of specific nuclear stains.

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Conflicts of interest
There are no conflicts of interest.

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