The Risk of Malignancy of Atypical Urothelial Cells of Undetermined Significance in Patients Treated With Chemohyperthermia or Electromotive Drug Administration

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BACKGROUND: Chemohyperthermia (C-HT) or electromotive drug administration (EMDA) are alternative therapies to radical cystectomy in patients with non-muscle-invasive bladder cancer who do not respond to intravesical therapy with bacille Calmette-Guérin. **METHODS:** The authors investigated a group of 87 patients with a diagnosis of high-grade non-muscle-invasive bladder carcinoma or carcinoma in situ. Of these, 45 patients received EMDA of mitomycin (EMDA/MMC) and 42 patients were treated with C-HT and mitomycin therapy (C-HT/MMC). In accordance with the Paris System for Reporting Urinary Cytology, a cytological diagnosis was made and patients with diagnoses of atypical urothelial cells (AUC), suspicious high-grade urothelial carcinoma (SHGUC), or high-grade urothelial carcinoma also underwent histological bladder biopsies. **RESULTS:** In accordance with the Paris System for Reporting Urinary Cytology, the AUC cases may have cytological features of SHGUC present on atypical degenerated cells. In analyzing the AUC group without the SHGUC cases diagnosed on the basis of degenerated urothelial cells, the authors found a significant association between the AUC category and a negative histological biopsy. The SHGUC group, including cases with a SHGUC diagnosis rendered on degenerated urothelial cells, was associated with high-grade urothelial carcinoma or carcinoma in situ ($P = .0269$ for patients treated with EMDA/MMC and $P = .0049$ for patients treated with C-HT/MMC). **CONCLUSIONS:** In the urine samples from patients treated with EMDA/MMC or C-HT/MMC, a diagnosis of SHGUC could be made even on degenerated urothelial cells when considering cellular degeneration as a "physiological" consequence of the treatment that involves either normal or neoplastic cells. ***Cancer Cytopathol*** **2018;126:200-6.** *© 2018 American Cancer Society.*

KEY WORDS: chemohyperthermia (C-HT); electromotive drug administration (EMDA); suspicious high-grade urothelial carcinoma (SHGUC); the Paris System for Reporting Urinary Cytology; urine cytology.

INTRODUCTION

The management of non-muscle-invasive bladder cancer (NMIBC) after transurethral resection of a bladder tumor consists of surveillance and intravesical therapy. For patients who do not respond to first-line intravesical bacille Calmette-Guérin (BCG) or who have high-risk features, radical cystectomy continues to be a recommended treatment.[1,2] The alternative developing treatment for patients with high-risk NMIBC includes a combination of intravesical chemotherapy either with hyperthermia, called chemohyperthermia (C-HT),[3] or with an electric current to enhance transepithelial drug penetration, defined as electromotive drug administration (EMDA).[4] At the end of every cycle of treatment, cystoscopy and urine cytology must be performed.

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In the current study, we selected patients with high-grade NMIBC who were nonresponders to BCG and had been treated with EMDA of mitomycin (EMDA/MMC) or C-HT and mitomycin therapy (C-HT/MMC). We examined the urine samples collected during cystoscopy at the end of the therapy cycle. After treatment, numerous degenerated urothelial cells were observed in these cases, and a diagnosis was made in accordance with the criteria of the Paris System for Reporting of Urine Cytology (TPS).

Moreover, we correlated these cytological results with histological biopsies to evaluate the impact of cellular degeneration on the cytological categories of TPS.

MATERIALS AND METHODS

We investigated a group of 87 patients who were admitted to the study institution from 2012 to 2014 with the histological diagnosis of high-grade NMIBC or carcinoma in situ (CIS) and underwent EMDA/MMC and C-HT/MMC. More specifically, 45 patients were treated with EMDA/MMC and 42 patients underwent C-HT/MMC. The male-to-female ratio was 5:1, with a median patient age of 75 years (range, 55-87 years). The patients signed a consent form describing the investigational nature of the protocol.

All C-HT was administered using a Synergo SB-TS 101.1 medical device (Medical Enterprises Group, Amsterdam, the Netherlands). Each administration consisted of 40 mg of MMC in 50 mL of saline solution and bladder wall hyperthermia at 42.5° to 45 °C for 60 minutes. Induction of C-HT and MMC combination therapy was administered for 6 consecutive weeks; there also were 3 weekly administrations at month 3 and month 6.

In the patients treated with EMDA, 40 mg of MMC dissolved in 100 mL of water was infused intravesically through a Foley catheter by gravity and retained in the bladder for 30 minutes; at the same time, 40 to 60 milliampere (mA)/second of pulsed electric current to a maximum of 20 mA was administered externally for 30 minutes[5] Two dispersive cathode electrodes were placed on the lower abdominal skin. The bladder then was emptied and the catheter removed. Such treatment was administered once per week for 3 weeks.

Cystoscopy and urine cytology were performed at the end of every cycle of treatment. The urine samples collected during cystoscopy were fixed immediately by the surgeons in a preservative solution. When these samples, which lack contamination of nonurothelial cells, are compared with the voided urine specimens, they usually have higher cellularity and the degeneration resulting from a delay in the fixation process appears to be reduced. The criteria of adequacy for this type of sample were established by TPS.[6]

All cytological samples were evaluated by 2 different pathologists (E.D.R. and F.P.) in accordance with the cytological criteria for TPS classification. We specifically analyzed: 1) the increase in the nuclear-to- cytoplasmic (N/C) ratio; 2) nuclear hyperchromasia; 3) irregular nuclear membrane (chromatinic rim or nuclear border); and 4) irregular chromatin (coarse or clumped).[6] Cells with an increase in the N/C ratio of > 0.5 were considered negative if no other abnormal features were present; the category of atypical urothelial cells (AUC) was reserved for the nonsuperficial and nondegenerated cells with an increase in the N/C ratio and with the presence of only 1 of the cytologic criteria described above. Atypical degenerated cells also were classified as AUC even if they demonstrated >1 of the previously mentioned criteria. According to TPS, a number of malignant cells of <10 cells or ≥ 10 cells, respectively, identify the categories of suspicious high-grade urothelial carcinoma (SHGUC) and high-grade urothelial carcinoma (HGUC). Malignant cells indicated urothelial nonsuperficial and nondegenerated cells with an increase in the N/C ratio of > 0.5, moderate to severe hyperchromasia, and 1 of the 2 cytological features (irregular clumpy chromatin or marked irregular nuclear membranes). Cellular degeneration is an exclusion criterion for SHGUC, and in cases presenting with a small number of urothelial cells (<10 cells) with malignant cytologic features having the appearance of degenerated urothelial cells, a diagnosis of SHGUC was not rendered and a final diagnosis of AUC was made.

Patients with a cytologically negative diagnosis for high-grade urothelial carcinoma (NHGUC) were followed using repeat urine cytology and the specimens were voided urine or bladder washing specimens obtained at the follow-up cystoscopy.

Surgeons performed bladder biopsies for all cases classified as NHGUC, SHGUC, or AUC.

The same pathologists examined the histological biopsies and an immunohistochemical approach using p53, cytokeratin 20, CD44, and Ki-67 was applied to distinguish between reactive urothelium and the recurrence of high-grade bladder carcinoma or CIS.

A third pathologist (L.M.L.) was consulted for cases with controversial diagnoses.

The urine specimens examined were prepared using the ThinPrep 5000 automated slide processor (Hologic, Marlborough, Massachusetts), fixed in 95% ethanol for 15 minutes, and stained using the standard Papanicolaou method following the manufacturer's instructions. It has been demonstrated that the ThinPrep method identifies nuclear and chromatin details better than conventional methods in urine citology.[7,8]

For the immunohistochemistry studies, the avidin-biotin-peroxidase complex method was performed on paraffin sections by applying technical procedures previously reported,[9] using a commercially available kit (Dako LSAB2; Dakopatts, Glostrup, Denmark) and the following commercially available monoclonal antibodies: cytokeratin 20, CD44, p53, and Ki-67.

RESULTS

In the patients treated with EMDA/MMC, we found that 15 of 45 samples (33.3%) were classified as NHGUC, 13 samples (29%) were classified as HGUC, 5 samples (11%) were classified as SHGUC, and the remaining 12 samples (26.7%) had a diagnosis of AUC.

In the group of patients treated with C-HT/MMC, 12 of 42 samples (28.6%) were classified as NHGUC, 10 samples (23.8%) were classified as positive for HGUC, 6 samples (14.3%) were classified as SHGUC, and 14 samples (33.3%) were classified as AUC.

The cases with a cytological diagnosis of NHGUC did not demonstrate any sign of clinical, cytological, or pathological recurrence of urothelial carcinoma over the course of the study, during the follow-up, and at the time of last follow-up.

The histological biopsies, which were performed only in patients with a cytological diagnosis of HGUC, SHGUC, or AUC, confirmed the cytological diagnoses of HGUC or CIS in all the cases in which patients were treated with either EMDA/MMC or C-HT/MMC. In 4 of 5 patients treated with EMDA/MMC (80%) with a cytological diagnosis of SHGUC and in 6 of 6 patients with SHGUC (100%) who underwent histological biopsy after C-HT/MMC therapy, a histological diagnosis of HGUC or CIS was made.

In the patients with cytology findings of AUC, the histological diagnosis was HGUC or CIS in 8 of 12 patients treated with EMDA/MMC (67%) and in 9 of 14 patients treated with C-HT/MMC (64%), respectively.

In the AUC cases in the current study, we identified a subset of samples consisting of 8 patients treated with EMDA/MMC and 9 patients after C-HT/MMC. This subset satisfied the criteria for SHGUC, but only in urothelial cells with cytological features of cellular degeneration. When analyzing the urine samples of these patients, we found numerous AUC and isolated urothelial cells degenerated with incomplete cytoplasm, discontinuous nuclear membranes, or large hyperchromatic nuclei and irregularly distributed clumpy chromatin or irregular nuclear membranes (Fig. 1). According to TPS, the cellular degeneration precludes a diagnosis of SHGUC and in these cases a diagnosis of AUC was made.

However, in separating these patients from the group of AUC cases, we found that the histological diagnosis of HGUC or CIS was made in 7 of 8 patients treated with EMDA/MMC (87.5%) and in 8 of 9 patients treated with C-HT/MMC (89%). In the remaining AUC cases, histological HGUC or CIS was observed in 1 of 4 patients treated with EMDA/MMC (25%) and in 1 of 5 patients treated with C-HT (20%).

Moreover, when analyzing the AUC group without SHGUC cases diagnosed on degenerated urothelial cells, we found a significant association between the AUC category and a negative histological biopsy in both patients treated with EMDA/MMC and those treated with C-HT/MMC; the SHGUC group including the cases with an SHGUC diagnosis rendered on degenerated urothelial cells was associated with HGUC or CIS ($P = .0269$ [odds ratio (OR), 33; 95% confidence interval (95% CI), 1559-6985] for patients treated with EMDA/MMC and $P = .0049$ [OR, 56; 95% CI, 2825-1110] for patients treated with C-HT/MMC) (Tables 1 and 2).

DISCUSSION

The treatment of patients with NMIBC encompasses a range of different procedures and interventions. Device-assisted intravesical chemotherapy regimens appear to demonstrate improved efficacy versus passive diffusion regimens. Studies comparing EMDA and C-HT with conventional treatment have yielded promising results in demonstrating the potential to improve intravesical therapy for patients with NMIBC.[10]

Cystoscopy and urine cytology collected at the end of cystoscopy were performed at the end of every treatment

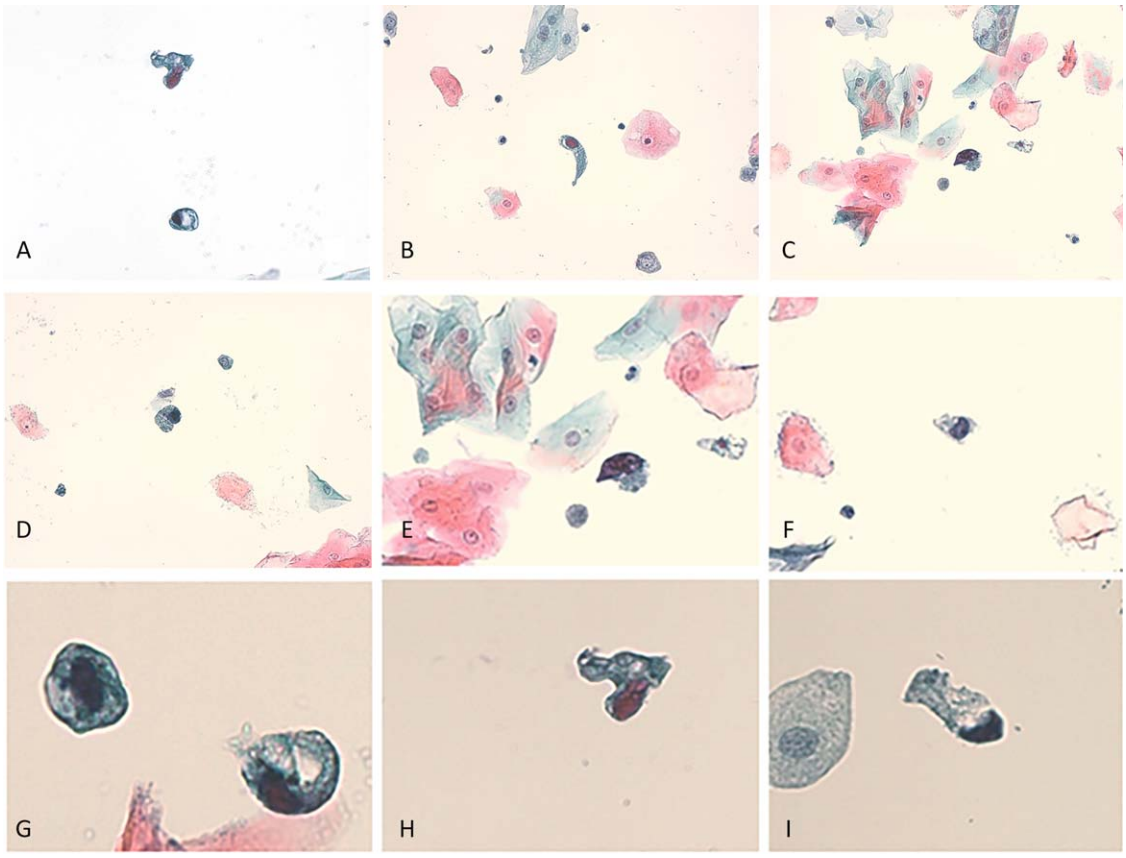

Figure 1. Atypical urothelial cells in the urine samples of patients after treatment with electromotive drug administration of mitomycin (EMDA/MMC) or chemohyperthermia and mitomycin therapy (C-HT/MMC). Single and degenerated cells in patients after EMDA/MMC or C-HT/MMC demonstrated an increased nuclear-cytoplasmic ratio, hyperchromasia, and an irregular nuclear membrane. In accordance with the Paris System for Reporting Urinary Cytology, in the presence of (A, B, D, E, F, and H) cellular degeneration as incomplete cytoplasm (original magnification × 400) or (C, G, H, and I) discontinuous nuclear membrane (original magnification × 400), a diagnosis of suspicious high-grade urothelial carcinoma should not be rendered.

TABLE 1. Correlation Between Cytological Diagnosis of SHGUC and AUC and Corresponding Histological Biopsy in Patients Treated With EMDA/MMC

Cytological Diagnosis	HGUC (Histological Biopsy)	NHGUC (Histological Biopsy)	Univariate Analysis *P*
SHGUC, no. (%) AUC, no. (%)	4 (80%) 8 (66.7%)	1 (20%) 4 (33.3%)	1
mSHGUC, no. (%) mAUC, no. (%)	11 (91.7%) 1 (25%)	1 (8.3%) 3 (75%)	P=.0269 (OR, 33; 95% CI, 1559-6985)

Abbreviations: 95% CI, 95% confidence interval; AUC, atypical urothelial cells; EMDA/MMC, electromotive drug administration of mitomycin; HGUC, high-grade urothelial carcinoma; mAUC, remaining atypical urothelial cells not considering modified suspicious high-grade urothelial carcinoma; mSHGUC, modified suspicious high-grade urothelial carcinoma (including diagnosis rendered on degenerated urothelial cells); NHGUC, negative diagnosis for high-grade urothelial carcinoma; OR, odds ratio; SHGUC, suspicious high-grade urothelial carcinoma.

TABLE 2. Correlation Between Cytological Diagnosis of SHGUC and AUC and Corresponding Histological Biopsy in Patients Treated With C-HT/MMC

Cytological Diagnosis	HGUC (Histological Biopsy)	NHGUC (Histological Biopsy)	Univariate Analysis *P*
SHGUC, no. (%) AUC, no. (%)	6 (100%) 9 (64.2%)	0 (0%) 5 (35.8%)	.26
mSHGUC, no. (%) mAUC, no. (%)	14 (93.3%) 1 (20%)	1 (6.7%) 4 (80%)	.0049 (OR, 56; 95% CI, 2825-1110)

Abbreviations: 95% CI, 95% confidence interval; AUC, atypical urothelial cells; C-HT/MMC, chemohyperthermia and mitomycin therapy; HGUC, high-grade urothelial carcinoma; mAUC, remaining atypical urothelial cells not considering modified suspicious high-grade urothelial carcinoma; mSHGUC, modified suspicious high-grade urothelial carcinoma (including diagnosis rendered on degenerated urothelial cells); NHGUC, negative diagnosis for high-grade urothelial carcinoma; OR, odds ratio; SHGUC, suspicious high-grade urothelial carcinoma.

cycle. These are most common methods used in evaluating the results of therapy.

For reporting urine cytology, we used TPS and, in accordance with this system, a cytological diagnosis was made on the basis of the following 4 categories: 1) NHGUC; 2) AUC; 3) SHGUC; and 4) positive for HGCU. The following 4 cytological criteria were considered at the cytological level: 1) increased N/C ratio; 2) nuclear hyperchromasia; 3) irregular nuclear membrane (chromatinic rim or nuclear border); and 4) irregular chromatin (coarse or clumped). The diagnosis of AUC was reserved for nonsuperficial and nondegenerated urothelial cells with an increased N/C ratio (>0.5) and the presence of only 1 of the following features: nuclear hyperchromasia, irregular nuclear membranes, and irregular coarse clumped chromatin.

For the diagnosis of SHGUC, 2 major criteria, such as an increase in the N/C ratio (at least 0.5-0.7) and moderate to severe hyperchromasia in nonsuperficial and nondegenerated urothelial cells, are needed and at least 1 of the cytological features such as irregular clumpy chromatin or marked irregular nuclear membranes is necessary as well. Quantitative criteria also were required and the diagnosis of SHGUC was limited to cases with a few severely abnormal cells (generally < 10).[11] In the SHGUC category, the cytological features must be evaluated in nondegenerated urothelial cells. In fact, the nuclei with cellular degeneration appear hyperchromatic and "blown-up"; the cytoplasm may be incomplete, resulting in a falsely increased N/C ratio and the nuclear membrane may appear irregular from dehydration.

In such cases, numerous degenerated urothelial cells frequently were observed and cellular degeneration can take the form of incomplete cytoplasm, poorly preserved chromatin details, or discontinuous nuclear membranes.[12]

The urine samples examined in the current study were collected during cystoscopy and these types of samples lack the contamination of nonurothelial cells when compared with voided urine samples and reduce the possibility of degeneration due to a delay in the fixation process. Thus, we can hypothesize that the degeneration noted in the urothelial cells in the samples in the current study mainly was caused by EMDA/MMC or C-HT/MMC.

When we considered the patients treated with EMDA or C-HT with a cytological diagnosis of AUC, we found that the percentage of histological HGUC or CIS in subsequent biopsies was 67% and 64%, respectively. These percentages were higher than the values reported in previous studies regarding the predictive ability of AUC for a subsequent histologic diagnosis of HGUC or CIS. It has been demonstrated that the risk of detecting a biopsy-proven HGUC or CIS after an AUC diagnosis ranges from 8.3% to 37.5%. In fact, the follow-up of patients with an AUC diagnosis has shown a wide ranging spectrum of "benign" conditions such as urolithiasis, cystitis, benign prostatic hypertrophy, renal disease, intravesical chemotherapy, or BCG immunotherapy.[11,13–18] In a recent study, Hassan et al demonstrated that the predictive values for the AUC, SHGUC, and HGUC categories of TPS were 53%, 83%, and 100%, respectively.[19]

In the AUC group of patients treated with EMDA and C-HT, we separated out the cases with a diagnosis of SHGUC made on the basis of urothelial degenerated cells and found that in this group the risk of histological HGUC or CIS reached 92.3% in patients treated with EMDA/MMC and 93.3% in patients after C-HT. In the remaining AUC cases, the risk of histological malignancy was 25% and 20%, respectively, which is very similar to the risk of malignancy for the AUC category of TPS.

Thus, we can hypothesize that the unusual risk of detecting HGUC or CIS at the time of biopsy after a cytological diagnosis of AUC in the patients treated with EMDA or C-HT in the current study could be explained by considering that this AUC group included cases with cytological features of malignancy that were observed on only a few "degenerated urothelial cells"; for such patients, according to TPS, a diagnosis of SHGUC cannot be made.

When we considered the SHGUC category including cases with a diagnosis of SHGUC rendered on degenerated cells, we found this group of patients, both those treated with EMDA/MMC and those receiving C-HT/MMC, were better than the group of patients with SHGUC excluding cases with cellular degeneration, a finding that correlated to subsequent biopsy proven HGUC or CIS ($P = .0269$ [OR, 33; 95% CI, 1559-6985] and $P = .0049$ [OR, 56; 95% CI, 2825-1110], respectively) (Tables 1 and 2).

The data from the current study appear to suggest that few and isolated urothelial degenerated cells with incomplete cytoplasm, hyperchromatic nuclei, irregular clumpy chromatin, or irregular nuclear membranes could be considered neoplastic cells and a diagnosis of SHGUC can be made.

The hypothesis that the cytological features defining the SHGUC category can even be used on degenerated urothelial cells and that cellular degeneration should not constitute an exclusion criterion from the SHGUC category in

TPS already was supported by a recent study by Deshpande and McKee regarding the impact of TPS on the diagnosis of HGUC.[20] In this article, the authors demonstrated that a cytological diagnosis of SHGUC, even in those cases with cellular degeneration, appears to improve the performance of TPS by decreasing the number of cases assigned to the AUC category and by improving its predictive accuracy for a subsequent histological diagnosis of HGUC.[20]

Moreover, it recently has been demonstrated that the N/C ratio, which is one of the major cytological criteria described in TPS and is strongly compromised in urothelial degenerated cells, also was imperfect in well-preserved urothelial cells demonstrating low interobserver reproducibility and numerous limitations.[21]

Cowan et al demonstrated that in a neoplastic specimen, a wide ranging spectrum of cytomorphologic changes exists and sometimes only rare cells satisfy all TPS criteria for a diagnosis of HGUC. Moreover, it was observed that in samples with a cytological diagnosis of HGUC, numerous neoplastic cells were degenerated and when analyzing the degenerative changes, the authors described urothelial cells with hyperchromatic nuclei, extremely irregular nuclear borders and condensed chromatin at the nuclear ridges or cells with an alteration in the N/C ratio as result of incomplete cytoplasm, well-preserved large hyperchromatic nuclei, and irregular nuclear membranes. According to TPS criteria, degenerated cells should be ignored. However, the authors concluded that hyperchromasia in giant cells with degenerative changes strongly suggests malignancy and therefore warrants a closer inspection of the specimen.[22]

The results of the current study suggest that a diagnosis of SHGUC could be rendered on urine samples collected during cystoscopy in patients treated with EMDA or C-HT, even on the degenerated urothelial cells, bearing in mind that the cellular degeneration might be a "physiological" consequence of the treatment that involves either normal or neoplastic cells. In such cases, further studies with ancillary testing methods including fluorescence in situ hybridization could clarify which cytological features are indicative of malignancy in degenerated urothelial cells.

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AUTHOR CONTRIBUTIONS

Conceptualization, methodology, and supervision: **Francesco Pierconti** and **Giovanni Schinzari**. Investigation: **Esther Diana Rossi, Patrizia Straccia, Guido Fadda, Francesco Pierconti**, and **Emilio Sacco**. Funding acquisition and resources: **Luigi Maria Larocca** and **Pier Francesco Bassi**. Writing–original draft: **Esther Diana Rossi** and **Francesco Pierconti**.

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