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*Original Article*

# Prevalence and spectrum of microsatellite alterations in nonmuscle invasive bladder cancers

Guillaume Legrand, Hany Soliman, Francis Dubosq, Jérôme Vérine, François Desgrandchamps, Hugues de Thé, Pierre Mongiat-Artus*, Guillaume Ploussard*

*Department of Urology and Paris 7 University, APHP, Saint-Louis Hospital, Paris, France; Department of Biochemistry and Paris 7 University, APHP, Saint-Louis Hospital, Paris, France; Department of Pathology and Paris 7 University, APHP, Saint-Louis Hospital, Paris, France; INSERM U944 UMR7212, Paris 7 University, Paris, France; and INSERM U728 - Institut Universitaire d'Hématologe, Paris 7 University, Paris, France. *These two authors contributed equally to this work.*

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**Abstract:** We aimed to identify interesting deleted chromosomal regions for bladder cancer diagnosis and carcinogenesis, and to evaluate the association between loss of heterozygosity (LOH) and clinico-pathological parameters. Microsatellite analysis was performed on urine sediment and tumor tissue from 43 consecutive patients with superficial transitional cell carcinoma (TCC) and from 42 consecutive controls. Informative cases were scored as LOH or allelic loss (AL) according to the decrease of the allelic-imbalance ratio. The prevalence of LOH and AL was 39.5% and 86%, respectively. Chromosome 9 was the most frequently altered, especially at 9p (35%). The total number of microsatellite alterations per analysis was correlated with age, grade, stade and EAU classification. The locus 17p13.1 was strongly associated with high-stage (p=0.01) and high-grade tumors (p=0.02). Specificity and sensitivity of LOH was 100% and 39.3% for diagnosis of malignant urinary disease. Specificity and sensitivity of AL was 73.8% and 88%, respectively. Allelic losses are a frequent and early event in bladder cancer, especially at 9p. Thanks to its high specificity, LOH may serve as a complementary tool for non invasive diagnosis of bladder cancer. Further study is warranted to evaluate the prognostic value of LOH on recurrence, progression and muscle invasion.

**Keywords:** Bladder cancer, microsatellite, loss of heterozygosity, allelic loss

## Introduction

Transitional cell carcinoma (TCC) of the bladder presents in both superficial forms, accounting for 80% of newly diagnosed cases- and invasive highly aggressive tumours. Even after complete transurethral resection, 50% to 75% of superficial tumours recur and up to 25% progress in stage or grade, even after a long tumor free period. Bladder cancer development is a complex multistep process and the pathway from normal to malignant urothelium is not clearly understood. Bladder tumor disease seems to occur in two different forms, one a slowly superficial disease with low risk of progression, and the other beginning as carcinoma in situ and having a more critical course with muscle invasion.

In addition to chromosomal instability, mutations in microsatellite sequences are a hallmark of neoplastic transformation and have been reported in several human cancers. In bladder cancer, loss of heterozygosity (LOH) has been described as a distinct and frequent type of molecular alterations [1-3]. The ability to identify clonal population of tumor-derived cells in urine sediment has been demonstrated [4]. Most of these series have evaluated microsatellite alterations as a non-invasive method for bladder cancer or recurrence detection in order to reduce follow-up cystoscopies and hence patients' discomfort [5-8]. LOH has been shown to involve several loci throughout the genome with chromosome 9 being the most frequently altered in both superficial and muscle-invasive bladder cancers [9]. LOH at 9q was often studied, contrary to 9p LOH, and various groups tried to identify putative tumor suppressor genes such as p53 and p16. Chromosomes 4,

6, 8, 11 and 17 are also regions thought to play an important role in urothelial carcinogenesis [10-11]. Altough hundreds of different microsatellite markers have been used for microsatellite alteration analysis, it is unclear how many markers and which chromosomal loci should used to evaluate LOH.

Microsatellite instability (MSI) that is primarly a feature of HNPCC-related tumors, also occurs in a subset of most sporadic cancers. It appears less common in bladder cancer but conflicting data have been reported in the literature [12-13].

The aim of the present study was to identify interesting chromosomal regions for bladder carcinogenesis, especially at 9p, and to evaluate the association between microsatellite alterations and clinico-pathological parameters. We analyzed for loss of heterozygosity (LOH) with respect to 20 microsatellite markers distributed on 12 different chromosomal arms. Sensitivity and specificity of this set, according to allelic-imbalance ratio were evaluated in a population presenting symptoms suggestive for bladder cancer [14-15]. This analysis was performed on the urine sediment and/or tumor tissue from 43 consecutive patients with superficial TCC and on urine sediment from 42 consecutive controls consulting for symptoms suggestive for bladder cancer.

## Materials and methods

### *Patients*

Microsatellite analysis was performed in a total of 85 patients. 43 patients enrolled in the study underwent a transurethral resection in our department for initial superficial TCC at the Saint-Louis Hospital (Paris, France) between January 2000 and November 2006. The mean age of patients at the time of TCC diagnosis was 62.8 years (range: 24.1 to 81.4). 59% were smokers. Tumor grading and staging were performed according to the 1973 World Health Organization grading system and the 1997 TNM classification guidelines. TCC were also classified according to the European Association of Urology (EAU) classification. Venous blood samples were withdrawn once from individual patients for constitutional DNA extraction. Microsatellite analysis was performed on tumor tissue and/or urine sediment obtained prior to each cystoscopic examination. Patients were routinely followed up according to the EAU guidelines. The distribution of cases was: 14 TaG1, 13 TaG2, 2 T1G2 and 13 T1G3. 25 of tumors recurred (58%) during a mean clinical followup of 34.4 months.

During the same period of time, a control group of 42 patients underwent microsatellite analysis, cystoscopy and urinary tract imaging because of symptoms suggestive for bladder cancer.

### *DNA extraction*

A representative fresh tumor biopsy was obtained intraoperatively by transurethral resection. Particular attention was paid to resecting a tumor sample not contaminated by healthy urothelial tissue. Approximately 1 hr prior to cystoscopy, voided urine samples (8-200 ml) and venous blood, as a source of constitutional DNA, were collected from each patient and coded. Urine samples were centrifuged at 800*g* for 10 min. The pelleted material, including exfoliated cells, was gently re-suspended in 3 ml of PBS and spun again at 800*g* for 10 min. This washing procedure was repeated once and finally the pellets were re-suspended in 0.5 ml of PBS. DNA was extracted from the wash urine suspension and from the corresponding blood samples using Qiagen Tissue and Blood Kits (Qiagen, Valencia, CA) according to the manufacturer's instructions. Aliquots were removed to measure DNA concentrations using the DNA-binding fluorochrome Hoechst 33258 (DyNA Quant 200, Hoefer Pharmacia Biotech, San Francisco, CA) before freezing at -20°C until use. Genomic control DNA of Family 134702 was the gift of Dr. H. Cann (Centre d'Etude de Polymorphismes Humains, CEPH, Paris, France).

### *Microsatellite analysis*

Microsatellite analysis was performed in 2 independent experiments to control the procedure's reproducibility and without knowledge of clinical and histological data. Two panels of 13 dinucleotide repeat sequences were used during the study and chosen by their performance on control DNA, informativity and frequency of LOH in bladder cancer [5,16]. 6 microsatellite markers were always used: 9p (D9S162, IFNA, D9S171, D9S1828), 5 (ACTBP2), 17 (IG P53). From January 2000 to August 2005, 7 markers completed the first set mapped to chromosomes 4

(D4S243, FGA), 11 (D11S1647), 14 (MJD52), 16 (D16S310), 17 (D17S695), 18 (D18S51). From August 2005 to November 2006, 7 others markers was used mapped to chromosomes 8 (D8S1820), 9q (ABL1), 11 (THO1), 13 (D13S153), 17 (D17S1879, D17S1820), 18 (D18S364). DNA of Family 134702 (CEPH, Paris, France) was used as control. The primer sequences used for PCR analyses (AB Applied Biosystems, Les Ulis, France) are available from authors upon request. For microsatellite analysis one primer of each marker pair was end-labeled with 1 of the following fluorophores: 6-FAM, NED® or HEX (AB Applied Biosystems) PCR was conducted essentially as described previously. Briefly, for each microsatellite marker, equal amounts of DNA (20 ng each of constitutional and urine-extracted) were subjected simultaneously in 2 different PCR tubes to 30-35 cycles of amplification in a Hybaid Touch Down thermocycler (Hybaid, Teddington, UK), as follows: 95°C for 30 sec, followed by the appropriate annealing temperature (52-60°C) for 1 min, an extension step at 72°C for 1 min and a final extension step at 72°C for 5 min. PCR products were analyzed on an ABI Prism 310 Sequencer (AB Applied Biosystems). This technique permits the estimation of allele size and the quantitative evaluation of allele ratio using GeneScan® and GenoTyper® software that were licensed from AB Applied Biosystems. Informative cases were scored as loss of heterozygosity (LOH) when the intensity of the signal for one allele was decreased more than 50% relative to the allele control. Allelic loss (AL) was scored if a minimal reduction of 20% in the allelic-imbalance ratio of the signals was calculated. In addition, the marker panel that defines MSI was the original Bethesda panel between January 2000 and November 2004, then the second National Cancer Institute panel since December 2004 using pentaplex PCR of quasi-monomorphic mononucleotide markers (BAT-25, BAT-26, NR-21, NR-24, NR-27) [14-15]. MSI was defined as the appearance of additional bands or band shifts.

*Statistical analysis*

The associations between LOH at individual loci and clinico-pathological parameters were tested with two-sided Fisher's exact test or chi-square test for qualitative data and with Student's t-test for quantitative data. The limit of statistical significance was defined as $p<0.05$. All statistical calculations were performed using SPSS 13.0 (Chicago, Illinois) software. The sensitivity and the specificity were determined for each test in urine sediment among patients with bladder cancer and controls (patients with only tumor tissue analyses were excluded).

## Results

A mean of 9.5 markers per analysis were informative (range: 5 to 13). Loss of heterozygosity (LOH) was detected in 17 patients, the overall frequency was 39.5%. The maximal number of LOH per analysis was 8. No analysis showed microsatellite alterations for all informative markers. The frequency of LOH varied considerably over all loci, ranging from 0% to 37.9% (**Table 1**). Despite their high heterozygosity, 6 microsatelite markers were altered in <10% of analyzed cases. All microsatellites on chromosome 9, in addition to those on 8p and 13q, were frequently altered, ranging from 18.8 to 37.9% of analyzed informative patients. LOH on chromosome 9p affected 35% of patients, and 25% of analyses showed 2 or more microsatellite markers on 9p altered. LOH on another chromosome was always associated with LOH on 9p.

The LOH frequency was lower among TaG1 (21.4%) and comparable among TaG2, T1G2 and T1G3: 46.2%, 50% and 53.8%, respectively.

The overall frequency of allelic losses (AL) was 86% (37 patients). Spectrum of alterations was similar to LOH concerning the chromosome 9. AL at 11p, 4q and 5pter-5qter were frequently observed. Only one marker was altered in <10% of analyzed cases.

The mean number of alterations (LOH) per loci and per patient was 0.144 (range: 0 to 0.7) and the total number of LOH and AL was significantly correlated with age, grade, stade and EAU classification, but not with smoking or recurrence during follow-up (**Table 2**).

To get additional information, associations between LOH at each individual loci and clinicopathological criteria were analyzed according to the same modelling (**Table 3**, representing the most sensitive markers). Correlation between microsatellite alterations and the respective grades and stages of the tumors was observed. The mononucleotide markers 17p13.1, 8p21.1

Table 1. Panel of microsatellite markers: locus, frequency of allelic losses, heterozygosity and informativity

| Marker | Site | LOH Frequency (%) | AL Frequency (%) | Heterozygosity (%) | No informative |
|---|---|---|---|---|---|
| D9S1828 | 9p31.3 | 37.9 | 51.7 | 76.3 | 29 |
| D9S162 | 9p22.1 | 29 | 43.5 | 72.1 | 31 |
| IFNA | 9p22 | 23.5 | 38.2 | 79.1 | 34 |
| ABL1 | 9q34.1 | 21.4 | 35.7 | 73.7 | 14 |
| D9S171 | 9p21.3 | 20 | 32.4 | 87.2 | 35 |
| D8S1820 | 8p21.1 | 18.8 | 31.3 | 84.2 | 16 |
| D13S153 | 13q14.2 | 18.8 | 18.8 | 84.2 | 16 |
| D11S1647 | 11p23.1 | 14.3 | 14.3 | 60.9 | 14 |
| IGP53 | 17p13.1 | 13.3 | 30.0 | 90.9 | 30 |
| THO1 | 11p15.5 | 13.3 | 46.7 | 78.9 | 15 |
| D17S1879 | 17q13.1 | 11.8 | 29.4 | 89.5 | 17 |
| ACTBP2 | 5pter-5qter | 11.4 | 34.3 | 92.1 | 35 |
| D16S310 | 16q21.33 | 10.5 | 21.0 | 65.5 | 19 |
| D18S364 | 18q21.2 | 8.3 | 8.3 | 63.2 | 12 |
| MJD52 | 14q32.1 | 8 | 24.0 | 89.3 | 25 |
| D18S51 | 18q21.33 | 4.2 | 29.2 | 82.8 | 24 |
| D4S243 | 4q33 | 0 | 34.8 | 82.1 | 23 |
| FGA | 4q31.3 | 0 | 20.0 | 86.2 | 25 |
| D17S1820 | 17q21.33 | 0 | 12.5 | 84.2 | 16 |

and 11p15.5 were associated with elevated age and high-stade and high-grade tumors, and the difference was strongly significant for the 17p13.1 marker. LOH at 9p31.3 was frequently present in TaG2 tumors with intermediate prognosis. No microsatellite marker was correlated with smoking or with recurrence during follow-up. The microsatellite marker 5pter-5qter was altered only in patients developing recurrence, but difference was not significant.

Analysis was performed on both urine sediment and tumor tissue in 32 patients. Results of both analyses were totally similar in 12 cases (37.5%). Tumor tissue was more sensitive than urine sediment to detect microsatellite alterations in 14 cases (43.8%), whereas urine sediment was more sensitive in 6 cases (18.8%). In one case, results of both analyses were different with LOH at 5pter-5qter detected in urine sediment and LOH at 13q14.2 in tumor tissue.

In the control group, LOH was detected in 3 patients only and revealed respectively a renal cell carcinoma, a bladder metastasis of colorectal cancer and a bladder neuroendocrine tumor. LOH concerned loci at 18q21.22, 17p13.1 and 5pter-5qter, but not the chromosome 9. All the remaining patients were free of tumor after investigations that revealed renal lithiasis in 2, cystitis glandularis in 2, urinary bilharzia in 1 and lupus nephropathy in 1. LOH specificity was 92.8% for diagnosis of transitional cell carcinoma (TCC) and 100% for diagnosis of malignant urinary disease, respectively. LOH sensitivity was very low (39.3%) for diagnosis of malignant urinary disease. The common odds ratio of LOH was 6.1 (95%CI [1.4-25.9], p=0.008).

Sensitivity and specificity of allelic losses (AL) were 88% and 73.8%, respectively. The common odds ratio of AL was 20.7 (95%CI [5.2-82.9], p<0.001).

## Discussion

We observed the prevalence of LOH to be approximately 40% that is lower that rates published in most series. This difference can be explained by the variation of microsatellite markers used (in type and in number) and by the ratio values retained to be indicative of LOH, essentially. The cut-off of the allelic-imbalance ratio varied from 20% to 50% [6,17-19] in the series or was not indicated. Interestingly, Schneider et al have calculated the cut-off value for each microsatellite at 2 SD to obtain a better sensitivity [16]. The prevalence of LOH seemed

Table 2. Number of microsatellite alterations per loci according to clinico-pathological parameters

| | Nb LOH per loci and per patient | p(ANOVA) | Nb AL per loci and per patient | p(ANOVA) |
|---|---|---|---|---|
| GRADE | | 0.07 | | 0.03 |
| I | 0.043 | | 0.182 | |
| II | 0.166 | | 0.361 | |
| III | 0.237 | | 0.435 | |
| | | 0.03 | | |
| I | 0.043 | | | |
| II/III | 0.2 | | | |
| STAGE | | 0.02 | | 0.02 |
| Ta | 0.088 | | 0.252 | |
| T1 | 0.252 | | 0.453 | |
| EAU staging | | 0.08 | | 0.07 |
| Low | 0.025 | | 0.173 | |
| Intermediate | 0.156 | | 0.337 | |
| High | 0.220 | | 0.411 | |
| | | 0.04 | | 0.03 |
| Low | 0.025 | | 0.173 | |
| Interm./High | 0.185 | | 0.370 | |
| AGE | | 0.02 | | 0.01 |
| <60 years | 0.054 | | 0.210 | |
| >60 years | 0.201 | | 0.408 | |
| Smoking | | 0.18 | | 0.02 |
| No | 0.185 | | 0.430 | |
| Yes | 0.093 | | 0.229 | |
| Recurrence | | 0.93 | | 0.30 |
| No | 0.147 | | 0.369 | |
| Yes | 0.141 | | 0.283 | |

to be correlated with this cut-off: 94-97%, 87% or 75% for a retained signal decrease superior to 30%, 40% or 50%, respectively [3,6-7,18-19]. The prevalence of AL (minimal reduction of 30% in the allelic-imbalance ratio) was in line with published values. It has become important to fix a single cut-off to be able to relate one series to another.

In addition, we solely included patients with superficial bladder carcinoma which may participate with the somewhat lower sensitivity in our study. Globally, comparison of a breakdown of the cohort in groups by stage of disease and by differentiation resulted in detection increasing with more advanced and aggressive disease [20].

The present study confirms the highest rate of LOH affecting the chromosome 9 [2-3,20-21]. Interestingly, allelic losses on chromosome 9p were more frequently observed than on chromosome 9q that was not consistent with recent findings [22-23]. Difference of prevalence of 9p LOH between low-stage/grade and high-stage/grade tumors was not significant (except at 9p22.1 concerning the stage). Even tumors of early stage and low grade displayed microsatellite alterations at chromosome 9 and tumors with intermediate grade exhibited the higher prevalence of 9p LOH, essentially at 9p31.3. Comparatively, LOH at 17p13.1 is strongly associated with high-grade, high-stage tumors and was not found TaG1/G2. The results confirm that chromosome 9 LOH and 17p13 LOH repre-

**Table 3.** Correlation between the presence of LOH at each locus and the clinico-pathologic characteristics and the oncologic outcome of patient cohort

| | ≥1 LOH % | 9p (n=12) % | 17p13.1 (n=4) % | 9p31.3 (n=9) % | 9p22.1 (n=7) % | 9p21.3 (n=5) % | 9p22 (n=6) % | 8p21.1 (n=3) % | 13q14.2 (n=3) % | 5pter-5qter (n=4) % | 11p15.5 (n=2) % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Smoking | | | | | | | | | | | |
| No | 50.0 | 40.0 | 27.3 | 45.5 | 30.8 | 40.0 | 25.0 | 25.0 | 50.0 | 18.2 | 50.0 |
| Yes | 30.4 | 26.8 | 5.9 | 25.0 | 20.0 | 60.0 | 16.7 | 18.2 | 9.1 | 5.0 | 0.0 |
| P value | 0.22 | 0.70 | 0.27 | 0.41 | 0.67 | 1.00 | 0.67 | 1.00 | 0.15 | 0.28 | 0.07 |
| Age | | | | | | | | | | | |
| <60y | 25.0 | 21.1 | 0.0 | 14.3 | 13.3 | 1.8 | 14.3 | 12.5 | 0.0 | 5.9 | 0.0 |
| >60y | 50.0 | 44.4 | 33.3 | 53.8 | 38.5 | 20.0 | 23.5 | 28.6 | 42.9 | 13.3 | 33.3 |
| P value | 0.10 | 0.17 | 0.02 | 0.05 | 0.20 | 0.65 | 0.66 | 0.57 | 0.08 | 0.59 | 0.17 |
| Stade | | | | | | | | | | | |
| Ta | 33.3 | 32.0 | 0.0 | 35.0 | 15.8 | 13.6 | 19.0 | 0.0 | 11.1 | 5.0 | 0.0 |
| T1 | 53.3 | 42.9 | 36.4 | 44.4 | 54.5 | 33.3 | 33.3 | 37.5 | 33.3 | 21.4 | 40.0 |
| P value | 0.21 | 0.51 | 0.01 | 0.69 | 0.04 | 0.21 | 0.42 | 0.20 | 0.52 | 0.28 | 0.11 |
| Grade | | | | | | | | | | | |
| 1 | 21.4 | 21.4 | 0.0 | 16.6 | 10.0 | 14.3 | 10.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 2 | 46.7 | 50.0 | 0.0 | 66.7 | 30.0 | 22.2 | 30.8 | 0.0 | 20.0 | 18.2 | 0.0 |
| 3 | 53.8 | 38.5 | 36.4 | 37.5 | 50.0 | 27.3 | 30.0 | 42.9 | 33.3 | 16.7 | 40.0 |
| P value | 0.19 | 0.31 | 0.02 | 0.06 | 0.14 | 0.72 | 0.45 | 0.12 | 0.44 | 0.34 | 0.09 |
| EAUstaging | | | | | | | | | | | |
| Low | 9.1 | 9.1 | 0.0 | 0.0 | 12.5 | 9.1 | 12.5 | 0.0 | 0.0 | 0.0 | 0.0 |
| Intermediate | 50.0 | 53.3 | 0.0 | 66.7 | 25.0 | 25.0 | 26.7 | 0.0 | 16.7 | 15.4 | 0.0 |
| High | 50.0 | 35.7 | 33.3 | 37.5 | 45.5 | 25.0 | 27.3 | 37.5 | 28.6 | 15.4 | 33.3 |
| P value | 0.06 | 0.06 | 0.03 | 0.01 | 0.27 | 0.55 | 0.70 | 0.16 | 0.56 | 0.46 | 0.12 |
| Recurrence | | | | | | | | | | | |
| No | 38.9 | 35.3 | 12.5 | 45.5 | 25.0 | 21.4 | 38.5 | 16.7 | 28.6 | 0.0 | 12.5 |
| Yes | 40.0 | 34.8 | 14.3 | 33.3 | 33.3 | 19.0 | 14.3 | 20.0 | 11.1 | 20.0 | 14.3 |
| P value | 1.00 | 1.00 | 1.00 | 0.70 | 0.70 | 1.00 | 0.23 | 1.00 | 0.55 | 0.12 | 1.00 |

sent two distinct pathways of tumor progression to high stage TCC, one being from Ta tumors and exhibiting 9p LOH [24-25]. Chromosome 9 losses occur early during urothelial tumorigenesis [9,26], whereas mutations in the p53 gene, located at 17p13, were commonly seen in high-grade tumors [27]. The invasive tumor population would be a combination of tumors with different backgrounds. Interestingly, LOH at 8p21.1 and 11p15.5 were also identified in this study as frequent progression-associated alterations [20].

No locus was correlated with tobacco carcinogens, LOH at markers studied may reflect a different way of carcinogenesis. Prevalence of LOH in the smoker subgroup was lower (30% versus 50% in the non-smoker subgroup, NS). These findings can limit the interest of microsatellite analysis as non-invasive method for screening smoker high-risk population.

In the majority of cases, results of tumor tissue were identical or more sensitive than urine sediment analyses. The lack of detection in urine sediment could be attributable to a limited amount of tumor cells in the collected urines and to the presence of a mixture of cancer, normal and inflammatory cells [8,23]. For about 20% of cases, allelic losses were detected in urine sediment but not in tumor tissue [3-4,23]. One possibility is that the urine sediment contained a more advanced tumor cells clones, which were not sampled during transurethral resection. Close surveillance is required for these patients because LOH may bear witness of flat lesions, such as dysplasia or carcinoma in situ that exfoliate easily. In this case the risk of recurrence within short delay seems to be probably high [7]. Furthermore, normal looking mucosa adjacent to or distant from urothelial tumor displays frequently genetic alterations [28-29]. In addition, tumors originating from the upper urothelial tract also may cause microsatellite alterations. The other hypothesis is that LOH in tumor specimen have been masked by cells of the normal mucosa.

Results showed the high specificity of LOH achieved by microsatellite analysis for diagnosis of superficial tumors in a population with symptoms suggestive for bladder cancer, in line with published studies [16]. Comparatively, application of urinary cytology is largely limited by low sensitivity and operator dependency, especially for low-grade tumors. Reducing the cut-off of the allelic-imbalance ratio provided better sensitivity, but a strong loss of specificity [30]. These results indicate that microsatellite analysis may serve as an important and complementary tool to cystoscopy for non-invasive diagnosis of bladder cancer. For the molecular detection of tumor cells, the interest is to provide the urologist with the highest sensitivity. Moreover the low sensitivity and some discrepancies between alterations observed in tumor tissue and urine don't suggest that a cystoscopic examination may be omitted if microsatellite analysis is negative. Nevertheless, the very high specificity makes microsatellite of urine sediment a good adjunct to cystoscopy and/or cytology. Presence of LOH as defined previously in our series must force the physician to look for malignant urinary tract disease. If investigations are negative, it is reasonable to propose that these patients suspicious for cancer lesions would benefit from careful follow-up. One of the interest of our series was that sensitivity and specificity calculations were performed among a population consulting for symptoms suggestive for bladder cancer, and not among healthy controls, as described previously [20].

Interestingly, LOH at chromosome 9 seemed to be specific of urothelial neoplastic origin whereas losses at chromosomes 5, 17 and 18 were detected in non-urothelial urinary tract tumors. However, no yet-defined set of modified markers can specifically identify cellular types or tumor localization.

Recently, van der Aa et al. have studied the utility of urine microsatellite analysis during the follow-up of bladder cancer patients [31]. They found that consecutive positive microsatellite analysis results were a strong predictor for future recurrences. In a 228 patient cohort, the risk to develop a recurrence at 24 months reached 83% if microsatellite analysis was persistently positive compared with only 22% when microsatellite analysis was persistently negative. However, in line with our results, they emphasized that sensitivity needed to be improved, for example, by patient selection and testing of additional genetic markers in urine samples.

Further study is warranted to evaluate the prognostic value of LOH on recurrence, progression and muscle invasion. Our group has recently

showed that LOH at 9p22 was a significant and independent predicting factor of global progression and progression to muscle-invasive bladder cancer [32]. Prognostic evaluation of a combination of different molecular alterations, such as LOH, FGFR3 and p53 mutations, seems to be interesting to better identify patients at high risk of progression and muscle invasion [33].

## Conclusions

Loss of heterozygosity is a frequent, important and early event in bladder cancer. Determination of a single cut-off value for allelic-imbalance is essential to compare and review several published studies. Reducing this ratio clearly improves sensitivity of microsatellite analysis, but cause a strong loss of specificity. The highest rates of alterations were located at the loci on chromosome 9, especially at 9p. LOH at 9p may reflect the existence of potential tumor suppressor genes in that chromosomal region such as p16 and contribute to the pathway to invasive disease of bladder cancer development.

**Address correspondence to:** Guillaume Ploussard, MD, Department of Urology and Paris 7 University, APHP, Saint-Louis Hospital, Paris, France. E-mail: g.ploussard@gmail.com

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