

Microsatellite instability and transitional cell carcinoma of the upper urinary tract

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INTRODUCTION

Upper urinary tract TCCs (UUT-TCC) are rare and account for 5% of all urothelial carcinomas [1]; 20–30% of the patients with UUT-TCC have a history of bladder TCC, but <2% with bladder TCC have UUT-TCC. The incidence of synchronous bilateral UUT-TCC is ≈3% and tumours of the renal pelvis are three to four times more common than ureteric TCC [2]. The standard treatment of UUT-TCC is radical nephroureterectomy, including removal of the bladder cuff at its distal extent. Whilst UUT-TCCs are relatively uncommon, there are few reported series including >50 patients [1].

Microsatellite instability (MSI) is characterized by alterations at the individual DNA nucleotide level, and is best seen in the microsatellite regions of DNA [3,4]. These are ubiquitous repetitive mono-, di-, tri-, tetra- and pentanucleotide repeat sequences, found in both exonic and intronic DNA. Whilst their function is unknown, they are useful markers of genetic instability and for linkage analyses [5]. MSI is described as a situation in which a germ-line microsatellite allele has gained or lost repeat units and thus had undergone a somatic change in length [3,6]. This type of alteration can be detected only if many cells are affected by the same change. So MSI is an indicator of the clonal expansion that is typical of a neoplasm; it is present in almost 15% of sporadic UUT-TCCs [4,5].

Significant advances have been made in managing UUT-TCC by considering MSI status, as a result of developments in molecular biology [4,6–8]. In this article, we review the current and future uses of MSI for following UUT-TCC.

GENETIC INSTABILITY

Tumour MSI indicates potential mutations or epigenetic alterations in mismatch repair (MMR) genes and is only detected if the same change affects many cells [3]. It is an indicator of the clonal expansion of neoplasms, and was first identified in tumours from patients with hereditary non-polyposis colorectal carcinoma (HNPCC) [3]. The detection of MSI, defined as expansion or deletion of one or more repeat units, provides objective evidence of genomic instability. MSI is an indication that genes encoding proteins involved in DNA repair have potentially been mutated. In addition, it seems that defective MMR genes, which fail to produce proteins that correct nucleotide mismatches during DNA replication, could increase mutations in other genes [3]. Distinct genetic profiles between upper and lower urinary tract tumours have been established [4,9]. Whilst MSI is rare in bladder cancer, as it is detected in ≈3% of TCCs, it occurs in >15% of UUT-TCCs (Table 1) [4,5]. Interestingly, it was suggested that certain clinico-pathological criteria are more common in UUT-TCC with MSI, e.g. location in the lower ureter, female sex, younger age and an inverted growth pattern [10]. Therefore, they could help pathologists to select which UUT-TCC may have MSI and may need to be screened (Fig. 1) [10].

Typical MSI of HNPCC is caused by deficient DNA MMR proteins and is defined with mono- and di-nucleotide repeat microsatellites. Paired DNA from tumour and normal tissues is extracted and amplified by PCR using microsatellite markers [4]. A panel of five such markers (Bethesda panel) is usually used to determine MSI status [11]. Two mononucleotide markers (BAT25 [4q12] and BAT26 [2p16]) and three dinucleotide markers (D2S123 [2p16], D5S346 [5q22] and D17S250 [17q]) are proposed. Whilst MSI is not solely a phenomenon of repetitive mono- and dinucleotides, this panel was found to be reproducible and representative of tumours

with MMR deficiency. In accordance with the National Cancer Institute workshop consensus on MSI, any pair of samples of normal DNA and tumour DNA that show instability at two or more loci of five is scored as high-frequency MSI. Conversely, a sample with no instability at any of the loci is scored as microsatellite stable. Any sample showing instability at one of the five microsatellite loci undergo a second test at that locus. If the instability is confirmed, additional loci, up to a maximum of 10, are tested to determine whether the phenotype of the sample is low-frequency MSI (one to three loci of 10) or high-frequency MSI (four or more loci) [11]. In 2004, the Bethesda guidelines were revised and are now even more accurate [12]. For low-frequency MSI tumours, further markers can be used, e.g. MFD15 (1q23), APC (5q22), BAT40 (1p13.1), D18S58 (18q22), D18S69 (18q21), D10S197 (10p12), MYC1L (1p34), UT5320 (8q24), ACTBP2 (6q13), CFS1R (5q33–q35), D20S82 (20p12), D11S488 (11q24), D9S242 (9q33). Conflicting reports of the MSI rates in different kinds of tumours have been published [4,9]. This variability may be partly a result of differences in the microsatellites studied [6,11,12]. More recently, a second variety of instability was identified [6], best seen at selective tetranucleotide repeats (elevated microsatellite alterations at selected tetranucleotides, EMAS) and seemingly related to p53 deficiency. Indeed, Ahrendt *et al.* [13] suggested that other than a mismatch repair pathway might be involved in the mechanism underlying microsatellite alterations in EMAS, which is probably related to abrogation of a p53-dependent repair pathway [13]. Whilst MSI occurs frequently in UUT-TCCs and is typical of HNPCC, EMAS does not seem to be related to this syndrome [6].

CYTOGENETIC ANALYSIS

Abnormal karyotypes are found in 66–80% of UUT-TCCs; indeed, cytogenetic analysis has

shown chromosome 7 trisomy, and the loss of chromosome 9, chromosome Y and chromosome 5q in UUT-TCC [14]. Like most human cancers, UUT-TCC develops through multiple and accumulated genetic aberrations that may lead to the inactivation of tumour-suppressor genes. The karyotypic profile of UUT-TCC is identical to that of bladder TCC, suggesting that the same pathogenetic mechanisms may underlie the process in both regions [14]. Many changes prevail but are not specific to this type of tumour (trisomy 7, -Y, or both). Microsatellite markers are extensively used to detect loss of heterozygosity at specific chromosomal loci that occurs in DNA close to putative tumour-suppressor genes [4].

UUT-TCC AND HEREDITARY TUMOURS

HNPCC is an autosomal dominant syndrome predisposing to colorectal cancer and which accounts for ≈5% of all colorectal cancers [3]. It is revealed by colorectal cancer (63%) or extracolonic cancers, most often of the endometrium (9%) or ovary, but sometimes of the UUT (5%), small bowel, stomach or hepatobiliary tract. However, the relative risk of UUT-TCC in patients with HNPCC is ≈14%[15]. Indeed, patients with HNPCC have a genetic risk of developing these cancers. HNPCC is caused by germ-line mutations affecting one or several MMR genes, i.e. *hMSH2* (60% of the time), *hMHL1* (30%) and more rarely *hMSH3*, *hPSM2* and *hMSH6* [3]. Patients with HNPCC having a colon cancer with high MSI levels no longer express the protein products of the *hMLH1* and *hMLH2* [3,16]. Applying the stringent clinical criteria for diagnosing HNPCC [17] (Amsterdam criteria, Appendix) shows that 6% of colorectal cancers are hereditary, and MSI screening identifies a further 4% of hereditary cancers [3]. In addition, the incidence of *de novo hMSH2* mutations is not negligible [8]. Subsequently, some hereditary cancers, whether of the colon or UUT-TCC, are misclassified as sporadic and their incidence underestimated [3,8]. Recently, in a study of patients with UUT-TCC who did not meet the international clinical criteria for HNPCC, it was established that systematic screening for MSI might help to detect hereditary disease, as for colorectal carcinoma [8]. Historically, MSI was first identified in tumours of patients with HNPCC, as high MSI levels are nearly always present in these tumours. Henceforth, routine screening for MSI in high-risk patients

TABLE 1 Clinicopathological criteria of the main series reporting MSI in urothelial carcinomas

Reference	Type of tumour	N microsatellites markers tested	N patients	Mean (range) age, years	High MSI rate, %	N HNPCC-related tumour markers
Roupret <i>et al.</i> [8]	UT-TCC	16	164	70 (54-90)	16	7
Amira <i>et al.</i> [4]	UT-TCC	5	24	68 (42-86)	22	3
Catto <i>et al.</i> [6]	UT-TCC	18	71	67 (54-85)	21	-
	Bladder	18 (EMAST)	89	67 (54-85)	14	-
Hartmann <i>et al.</i> [5]	UT-TCC	6	73	71 (37-85)	21	9
Bonnal <i>et al.</i> [9]	Bladder	6	33	66 (47-82)	0	-

with UUT-TCC should be recommended (Fig. 1) [7,8]. In a recent study, 164 patients treated for sporadic UUT-TCC were screened for MSI [8]; 27 (16%) had high MSI levels. For these patients the presence of a mutation was significantly related to a history of a HNPCC-associated cancer, and UUT-TCC occurrence before 60 years of age [8]. The mean age at diagnosis of UUT-TCC is 65-70 years; to target hereditary cancers, all patients with a high MSI level and who meet one of the Amsterdam criteria, or are aged < 60 years, should be tested for germline mutations [8]. Conversely, in cases of absent or low-level MSI, the test is not justified. Although screening for mutations of *hMSH2* and *hMLH1* is now available in many centres, it needs to be restricted to selected patients, as DNA sequencing is complex and expensive. Mutations in *hMSH2* account for 60% of HNPCCs and mutations in *hMLH1* for 30%. Detection of mutations in other genes (*hMSH3*, *hPSM2* or *hMSH6*) is not currently available as a routine. When gene mutations are detected, the patient benefits from multidisciplinary management [3,8]. The presence of other HNPCC-associated cancers is sought, patients are closely monitored, and genetic counselling is provided to the patient's relatives. Searching for MSI is thus a useful means of detecting hereditary cancers, and a screening test for MSI seems warranted in all patients with UUT-TCC and clinicopathological criteria, as for colorectal cancers (Fig. 1) [8].

MSI DETECTION BY IMMUNOHISTOCHEMISTRY (IHC)

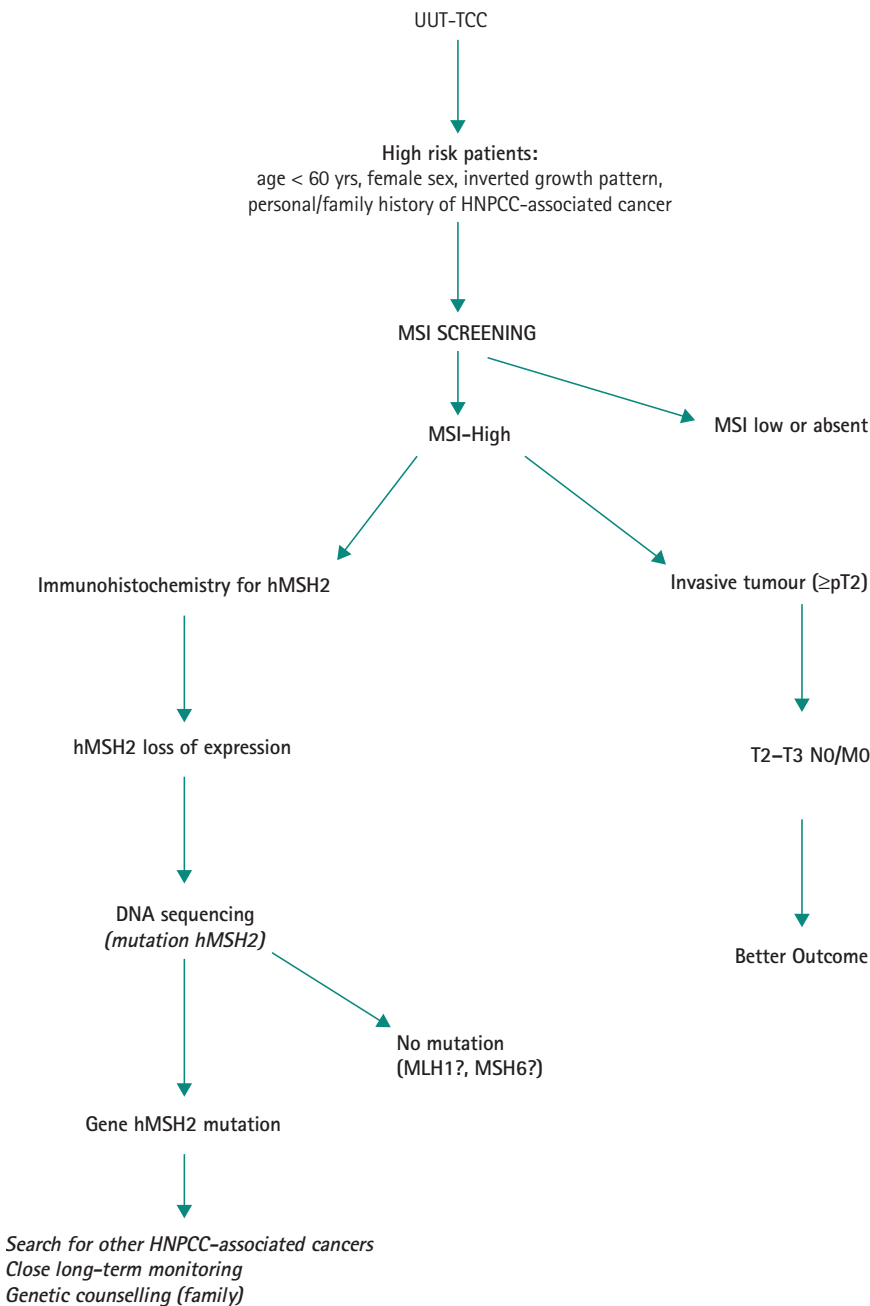
Some authors consider that in patients with colorectal cancer, the result of MSH2 and MLH1 IHC is sufficiently well correlated with MSI phenotype to act as a surrogate for MSI determination, especially as it is a quicker and

cheaper method [3,16]. Several publications have shown the high correlation between MSI phenotype determined by PCR and complete absence of MSH2 and MLH1 expression by using IHC [3,16]. However detecting MSI by PCR remains the standard for determining the MSI phenotype, as it is more specific for DNA repair gene changes than IHC [4,7,8]. IHC is a simple method but does not always detect protein loss. For instance, when other MMR system genes, e.g. *hMSH3*, *hPSM2* or *hMSH6*, are responsible for MSI tumour, the expression of MLH1 and MSH2 proteins could mislead the diagnosis. The results depend on the type of mutation or on DNA promoter hypermethylation [8]. Moreover, some cases of protein loss could be related to a gene germline mutation caused by large rearrangements or deletions that could be missed. Consequently, when IHC reveals loss of *hMSH2* protein expression in patients with a high MSI status and early-onset diagnosis of UUT-TCC, or history of tumour related to the HNPCC spectrum, the result is followed up by DNA sequencing to detect germline mutation. Therefore IHC is a useful additional test to show which MMR gene may be involved and whether DNA sequencing is necessary [8].

INVASIVE UUT-TCC AND SURVIVAL

UUT-TCCs infiltrating the UUT wall have a very poor prognosis; the survival at 5 years is less than half for stage T2-T3 tumours and < 10% for T4 or N+/M+ tumours [1]. The prognostic factors of survival which have been identified are patient age, tumour stage and, in some studies, tumour grade [1]. Ureteric tumours seem to have a worse prognosis than renal pelvic tumours [2]. Other pathological or molecular factors, e.g. positive lymph nodes or p53 and Bcl-2 overexpression, are closely

FIG. 1. The flow-chart for managing sporadic UUT-TCC.



related to tumour stage and are not considered as being independent factors [1,7].

It is well established that MSI is a prognostic factor independent of clinical stage in colorectal cancer, reflecting a low-grade tumour and longer survival [3,16]. Equally, it was shown that MSI status is a useful additional predictive factor in invasive UUT-TCCs. The survival of patients whose tumours have high MSI is better than that of patients

whose tumours do not. Furthermore, high MSI was identified as an independent prognostic factor on multivariate analysis. Indeed, in patients with an intermediate tumour prognosis (T2-T3/N0/M0), MSI successfully distinguished between patients with a better or poorer prognosis. Nonetheless, in patients with highly invasive tumours (T4 and/or N+/M+) whose prognosis is poor from the outset, MSI screening is unhelpful. The mean age of patients with UUT-TCC is 71 years at

presentation, but high MSI is not correlated with patient age, as the mean age of those with high MSI is 72 years. Univariate analysis of survival in all patients showed that age, stage, grade and MSI status had a significant effect. The mean (SD) overall survival was significantly longer in patients whose tumours had high MSI than low/absent MSI, at 37 (22) vs 21 (15) months ($P = 0.003$). When only those variables that appeared significant in the univariate analysis were entered into the final models of the multivariate analysis, only stage, age and high MSI were independent prognostic factors ($P = 0.023$, 0.01 and 0.022, respectively) [7].

FURTHER DEVELOPMENT

Although most patients currently undergo nephroureterectomy as the standard treatment for UUT-TCC [7,8] conservative surgery is increasingly being advocated for superficial UUT-TCC or invasive UUT-TCC with a good prognosis [18]. Patients aged < 71 years with a T2 tumour and high MSI might benefit from nephron-sparing surgery in the near future. Moreover, MSI is also used to decide whether patients with colorectal cancer should receive adjuvant chemotherapy [16]. Indeed, cell lines with high MSI respond less favourably to chemotherapy than do stable cell lines. This might also be the case for invasive UUT-TCC, which needs further investigation.

As reliable predictors of tumour diagnosis or recurrence are critically important for managing UUT-TCC, the diagnostic use of noninvasive urinary detection of MSI is currently under development. The successful molecular diagnosis of UUT-TCC and bladder tumours by detecting genetic lesions (loss of heterozygosity or MSI) in exfoliated cells from urine was reported by several groups [19,20]. A large multi-institutional study is currently underway in the USA to assess the accuracy of the diagnostic use of the urinary detection of MSI.

CONCLUSION

The MSI test should be used in every high-risk patient diagnosed with UUT-TCC, as it is positive in 16% of them. If the MSI level is high, hereditary cancer may be suspected, in particular if the patient is aged < 60 years or has a personal or familial history of HNPCC-

related cancer. High MSI also has prognostic value, particularly for patients aged <71 years and with a T2/T3/N0-M0 tumour, allowing the identification of a subset of patients who have better survival and may require more stringent follow-up. To help in managing patients with sporadic UUT-TCC, we propose the decision flow-chart (Fig. 1).

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CONFLICT OF INTEREST

None declared.

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Abbreviations: UUT, upper urinary tract; MSI, microsatellite instability; MMR, mismatch repair; HNPCC, hereditary non-polyposis colorectal carcinoma; EMAST, elevated microsatellite alterations at selected tetranucleotides; IHC, immunohistochemistry.

APPENDIX

The Amsterdam Criteria I and II [17]
Amsterdam criteria I.

- One member diagnosed with colorectal cancer before age 50 years
- Two affected generations.
- Three affected relatives, one of them a first-degree relative of the other two.
- Familial adenomatous polyposis should be excluded.
- Tumours should be verified by pathological examination.

Amsterdam criteria II:

- There should be at least three relatives with an HNPCC-associated cancer (colorectal cancer, cancer of the endometrium, small bowel, ureter or renal pelvis).
- One should be a first-degree relative of the other two.
- At least two successive generations should be affected.
- At least one should be diagnosed before age 50 years.
- Familial adenomatous polyposis should be excluded in the colorectal cancer cases.
- Tumours should be verified by pathological examination.