Gross examination of bladder specimens

M C Parkinson, C Fisher

Introduction
Bladder specimens received by pathologists commonly include endoscopic biopsy specimens and tissue from transurethral resections (TURB), both of which incorporate subepithelial tissues to a varying depth, as well as those resulting from cystectomy, cystoprostate-urethrectomy and pelvic exenteration. Less frequently seen are full thickness specimens of the bladder wall carried out at laparotomy ("open" biopsy), diverticula, and partial cystectomy specimens such as those for urachal carcinoma in which bladder dome, urachus, and umbilicus are excised. An Endocut needle based on the Trucut principle is currently being assessed for the ease with which information on tumour invasion can be derived from the tissue specimen.

The commonest indication for the examination of bladder tissue remains the diagnosis and assessment of prognostic factors in transitional cell carcinoma (TCC) which comprises 95% of bladder cancer in Britain.

Diagnosis and management of carcinoma
In addition to establishing the diagnosis, the aims in selecting and processing specimens from patients with suspected bladder cancer are: (1) to detect the risk factors indicating further disease activity or progression; and (2) to identify possible features associated with response to different forms of treatment. The risk factors vary according to tumour stage (tables 1, 2, and 3); established clinical factors have been included to emphasise the importance of pathology in management.1-3

"Superficial disease" meaning tumour confined to the transitional epithelium or lamina propria (table 4) is differentiated from "muscle invasive tumours"—those invading the muscularis propria. The former is a clinical rather than a pathological term but is widely used in practice and publications to distinguish patients with the two principal forms of tumour which have different courses and therapeutic possibilities. Tables 1, 2, and 3 represent composite lists derived from a number of publications in which the weight of each factor varies.

Of features influencing treatment, only squamous metaplasia in muscle invasive carcinoma used in some centres as an indicator that the tumour will fail to respond to radiotherapy.

The staging and grading systems referred to throughout this broadsheet are based on those proposed by the International Union against Cancer (UICC) in 19784 and not the more recent proposals of 1987.5 The latter have provoked criticism and concern because of the potential for confusion regarding what constitutes an adequate biopsy specimen for stage assessment and the failure to subdivide pT4 into stages known to have differing prognoses.6 The 1978 UICC classification is the system adopted by the Medical Research Council Working Party in Urological Cancer, the European Organisation for Research on the Treatment of Cancer, and is the classification preferred by the British Journal of Urology for publication.7,8

The UICC 1978 post-surgical histopathological classification and the modifications used in the St Peter's Group of hospitals are shown in table 4. The division of UICC stage pT1 into pT1a (invading papillary cores) and pT1b (infiltrating lamina propria) has prog-

---

Table 1 Predictors of recurrence in superficial TCC

<table>
<thead>
<tr>
<th>Factor</th>
<th>Categories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of tumours</td>
<td></td>
</tr>
<tr>
<td>Size &gt; 5 cm</td>
<td></td>
</tr>
<tr>
<td>Depth of invasion</td>
<td></td>
</tr>
<tr>
<td>Histological grade</td>
<td></td>
</tr>
<tr>
<td>Coexistent carcinoma in situ/dysplasia</td>
<td></td>
</tr>
<tr>
<td>Epidermal growth factor receptor positivity</td>
<td></td>
</tr>
<tr>
<td>Recurrence at three month follow up cystoscopy</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Predictors of progression (increased stage) in superficial TCC

<table>
<thead>
<tr>
<th>Factor</th>
<th>Categories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of tumours</td>
<td></td>
</tr>
<tr>
<td>Tumour size</td>
<td></td>
</tr>
<tr>
<td>Depth of invasion</td>
<td></td>
</tr>
<tr>
<td>Histological grade</td>
<td></td>
</tr>
<tr>
<td>Coexistent carcinoma in situ/dysplasia</td>
<td></td>
</tr>
<tr>
<td>Epidermal growth factor receptor positivity</td>
<td></td>
</tr>
<tr>
<td>Loss of surface blood group antigens</td>
<td></td>
</tr>
<tr>
<td>Aneuploidy</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Muscle invasive TCC: histological prognostic factors (urovial)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Categories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour size</td>
<td></td>
</tr>
<tr>
<td>Histological grade</td>
<td></td>
</tr>
<tr>
<td>Pattern of invasion</td>
<td></td>
</tr>
<tr>
<td>Lymphatic invasion</td>
<td></td>
</tr>
<tr>
<td>Nodal state</td>
<td></td>
</tr>
</tbody>
</table>

---
Table 4 Definitions of superficial and invasive disease used by UICC (1978) and St Peter’s Hospitals

<table>
<thead>
<tr>
<th>UICC 1978*</th>
<th>Modification used at St Peter’s Hospitals</th>
</tr>
</thead>
<tbody>
<tr>
<td>No evidence of tumour found</td>
<td>pT0</td>
</tr>
<tr>
<td>Preinvasive carcinoma (carcinoma in situ)</td>
<td>pT1S</td>
</tr>
<tr>
<td>Papillary non-invasive carcinoma</td>
<td>pTa</td>
</tr>
<tr>
<td>Tumour not extending beyond lamina propria</td>
<td>pT1</td>
</tr>
<tr>
<td>Tumour invasion of superficial muscle (not more than halfway through muscle coat)</td>
<td>pT2</td>
</tr>
<tr>
<td>Tumour invading deep muscle (more than halfway through muscle coat or with invasion of perivesical tissue)</td>
<td>pT3</td>
</tr>
<tr>
<td>Tumour invading prostate or other extra-vesical structures</td>
<td>pT4</td>
</tr>
<tr>
<td>The extent of invasion cannot be assessed</td>
<td>pTX</td>
</tr>
</tbody>
</table>

*Terms used by clinicians

nocastic value. Recent work has suggested that tumours deep to the muscularis mucosae but not invading the muscularis propria have a worse prognosis than more superficial tumours and thus a third prognostic pT1c subgroup may be justified. Division of pT3 into pT3a (invasion of deep muscle) and pT3b (infiltration of perivesical adipose tissue) represents an abbreviated form of a description of invasion that pathologists would naturally record. Precise assessment of the extent of muscle invasion, however, is not usually possible in biopsy material and it is acknowledged that diagnosis of pT2 and pT3a and b may only be confidently made on cystectomy specimens. Division of pT4 into pT4a (invading prostate, uterus, or vagina) and pT4b (invading pelvic or abdominal wall) reflects the clinical staging. Further division of pT4a into pT4a1 (invading prostate ducts and acini) and pT4a2 (infiltrating prostatic stroma) also has prognostic value. Despite the aforementioned histopathological divisions it is reiterated that clinicians commonly refer to pTis, pTa, and pT1 as ‘superficial disease’ and reserve the term ‘invasive’ for pT2–pT4.

Bladder biopsy and transurethral resection (TURB)

A superficial papillary carcinoma measuring only millimetres in diameter will be subjected to excision biopsy using ‘cold’ cup forceps such as Storz, diathermy forceps, or a small diathermy loop. In the case of larger neoplasms assessed clinically as not invading muscle, TURB is carried out using a diathermy loop producing strips of tissue 6 mm in diameter and of variable length. This may be followed by resection or biopsy of the muscle base, the result being delivered as a separate specimen so that the completeness of the resection can be assessed.

When tumours are clinically staged at presentation as invading muscle a similar local resection may be performed or a biopsy (using the instruments referred to above) may be done to confirm the diagnosis before planning definitive treatment.

In addition to tissue from the main tumour, any red or velvety areas of urothelium are sampled as such appearances may represent flat preinvasive malignancy. At the cystoscopic diagnosis of the first tumour, biopsies are commonly taken from macroscopically normal urothelium, away from the tumour site, to allow the presence of flat preinvasive malignant lesions to be diagnosed and their extent estimated. These are often referred to as ‘random biopsies’, but ideally should be from predetermined specific sites in four vesical quadrants. In some centres especially in patients with high grade papillary lesions or flat carcinoma in situ (CIS), biopsy specimens of the urethra and prostate are submitted for assessment of surface and ductal urothelium.

Pathologists should acquaint surgeons with the common source of artefacts which are: mechanical, as tissue is transferred from biopsy forceps to fixative with the aid of a gauze swab, and thermal, if overheating occurs in tissue taken with a diathermy loop. Epithelium which is the site of CIS is especially easy to detach, partially or completely. Fixation is achieved in formal saline.

REPORTING

In the macroscopic description the site of origin of the specimen given by the surgeon should be reiterated. In this context some hospitals with large urology units find it useful to have a diagrammatic stamp of the bladder in theatre (fig 1) to enable surgeons to indicate the site of origin of the specimen. In this respect it should be noted that the anatomical locations used by surgeons (figs 1 and 2) may not be identical with those a pathologist would apply. There is agreement on the trigone and lateral walls, but the extensive posterior wall, vault/dome, and restricted anterior wall are still recognisable if figure 2 is turned through 90° to the left—it is, into the cystoscopic position. The description of cup biopsies includes the number, size, and presence of a papillary lesion. These specimens should be transferred to cassettes with minimal handling. For TURBs it is useful to give a measurement of the aggregated tissue mass. Specimens up to 6 g (or occupying three cassettes) should be processed in their entirety; above this quantity laboratories may find it necessary to take only a representative sample,
in which case an effort should be made to select those containing muscle and the proportion of the specimen processed for examination stated. If muscle is not identifiable it is preferable to process all the material.

The **microscopic** report on biopsy specimens must record the tissues present—urothelium is frequently shed when it is the site of flat preinvasive malignancy, thus a report of "denuded biopsy" is significantly different from "no tumour seen". Similarly, invasion of the lamina propria may have different implications depending on the presence or absence of attached muscularis propria. Care must be taken to distinguish the thin attenuated and often incomplete muscle layer within the lamina propria (muscularis mucosae) from the muscularis propria. Grade, stage of tumour (see above), and a comment on vascular/lymphatic invasion are given, and in the case of random biopsy specimens the number showing dysplasia or CIS. Similar facts are supplied for TURBs, but depth of muscle infiltration is often impossible to assess and the only realistic statement becomes "stage P2 at least". Adipose tissue in biopsy specimens is not a clear indication of extravesical sampling as it may be present within the muscle layers.

**Partial cystectomy**

These specimens, including those for urachal carcinoma, are best fixed pinned out flat on a cork board inverted into a tank of formalin. The **macroscopic** description includes the size and thickness of the bladder disc and a description of the lesion. Blocks are then selected to include the deepest tumour level, the junction between tumour and normal tissue, and the excision limits. With respect to **urachal carcinoma**, blocks can also be selected from the urachus if patent or cystic, and when not visible macroscopically tumour may be apparent microscopically in random blocks from the connective tissue cord between bladder disc and umbilicus (fig 2). Metaplasia and dysplasia may be seen in the urachal remnant when carcinoma develops at this site.

**Macroscopic description of diverticulectomy** specimens includes a measurement of the diameter and appearance of the urothelium with a special regard to white areas (probably keratinising squamous metaplasia) and tumour. Tumours are sampled as described above and blocks are selected from the neck of the diverticulum to assess preinvasive malignancy. From diverticula without tumours random blocks are taken to assess metaplasia, inflammation, and preinvasive malignancy.

**Cystectomy**

These specimens are sometimes opened in the fresh state and pinned out, but inflation of the intact specimen with formalin and submersion in fixative is strongly recommended as it preserves the anatomy (fig 3). This is easily achieved in theatre by the surgeons. Ureters and urethra are occluded by ties or stitches as are occasional vesical punctures and the bladder is injected with formalin using a dark green needle and 25 cc syringe. Both vesical and urethral urethelial fixation are improved if catheters are removed before distension. After 24 hours of fixation the occlusive ties or sutures are removed from the urethra and a sound is passed into the bladder. Using a large knife and the sound as a guide, an incision is made along the length of the urethra and continued into the bladder anterior to the ureteric orifices (fig 2), thereby dividing the specimen into anterior and posterior portions (fig 4). Photographs can be taken at this stage if required. Ureters are opened from the resection margin into the bladder, or when ureteric stumps are short and not apparent the intramural ureters can be traced from the ureteric orifices. These are commonly slit-like and may be difficult to see but their position at the limits of the interureteric bar is a useful guide (fig 1). Some may prefer to take blocks from the ureteric limits and the ureterovesical junctions before opening those areas of the ureter (see below).

The **macroscopic** description should include the nature of the specimen—presence or absence of the urethra and details of the uterus and adnexa in the female. A remark is made on the distension or otherwise of the bladder by formalin because anatomical landmarks and tumour may be difficult to see in the collapsed fixed bladder and anatomical sampling will not be exact. The length, external diameters and wall thickness of the terminal ureters, the patency of ureteric orifices and the diameter of the prostate are noted. The appearance of any neoplasms (ulcerating, nodular, papillary), their site, size, and depth of infiltration of the wall are recorded. Similarly, neoplasms in the ureters or urethra are noted and prostatic disease may be suspected from the presence of cream coloured tissue in a linear ductal distribution irradiating from the urethra, or, as a mass effacing the architecture of the gland. The size and appearance of lymph nodes in pelvesical adipose tissue is described. Presence or absence of tumour extension to the seminal vesicles can be noted as blocks are selected (as described below).

It is helpful to view **sampling** in terms of deriving facts at two levels—those essential
Gross examination of bladder specimens

For staging, prognosis, and management and others which give additional valuable information from a more detailed standardised approach in an academic unit (fig 5). The sampling essential for clinical management includes tissue from the tumour (or tumours), incorporating the greatest depth of invasion and a coronal prostatic block which will include urethral urothelium. Blocks of the terminal ureters may be transverse or longitudinal and allow invasive or preinvasive malignancy to be assessed which may correlate with later development of upper tract tumours. A section of urethra permits diagnosis of carcinoma or "Pagetoid" spread which may be associated with further development of the latter on the glans or vulva. If surgery has been limited to cystoprostatectomy, sampling of flat urothelium away from the tumour is required to assess CIS, as multifocality of disease (papillary or flat) implies a higher risk that the patient will develop further tumour in the residual urethra. A block from the ureterovesical junction (UVJ) (see below) is essential if the ureter is dilated and its orifice patent because the incidence of invasive tumour in the intramural ureter is likely to be high. The authors have also found it invaluable to take blocks from both lateral walls, trigone, dome, anterior wall, anterior urethra, prostate and seminal vesicles. Blocks from the UVJs can be obtained by passing a sound into the ureter and incising the bladder on both sides of the ureter so that the latter is removed as a cylinder with a strip of bladder (fig 6). A parasagittal section of prostate should include ejaculatory duct and seminal vesicle both of which may be affected in transitional carcinoma (fig 7).

In women a sagittal or parasagittal block through the trigone and posterior urethra to the vaginal wall will demonstrate extension of tumour to the genital tract.

Microscopic reports will include grade, stage, vascular/lymphatic invasion, a statement on multifocality of papillary or invasive carcinoma and CIS, and the presence or absence of malignancy in the ureteric and urethral resection edges.

For purposes of research and audit it is helpful to use the P (procedure), SNOMED code so that numbers of biopsy and cystectomy specimens can be easily assessed. For clinicopathological studies access to the first tumour is frequently essential and the M code may be modified to note this fact.

Special techniques

Immunohistochemistry for epithelial antigens can be carried out on formalin fixed, paraffin wax material. Some centres, however, use special additional techniques to assess the

---

**Figure 3** Biected bladder after distension with formalin. The trigone and posterior wall are shown on the right; the anterior wall covered by tumour is shown on the left.

**Figure 4** Bladder bisected by the incision shown in fig 2 producing the cut surfaces illustrated in fig 3.

**Figure 5** Cut surface of posterior half of bladder following incision shown in figs 2 and 4—sites of blocks are indicated. Additional informative blocks when indicated: Uretero-vesical junctions, parasagittal prostate with ejaculatory duct and seminal vesicle. Sample: trigone, posterior, lateral, anterior wall and vaults.
prostate
eosin).
and
in
orifice
site
the
vesicle
extending through
Figure
(arrowed) (haematoxylin

6
894
Uretero-vesical junction, dilated ureter on the right and bladder wall on the
left. Invasive tumour is present in the terminal intramural course and at the orifice
(haematoxylin and eosin).

895
cytogenetics laboratory. Flow cytometry for
the detection of aneuploidy, the presence of
abnormal stemlines, and cell cycle analysis (S
phase fraction as a measure of proliferative
activity) can be carried out on bladder wash-
ings, fresh tissue, or on paraffin wax sections,
and each laboratory will have its preferred
technique. Fresh tissue may also be required
for immunohistochemical demonstration of
EGF receptors, and for in situ hybridisation
techniques.13 Obviously, with small biopsy
specimens there may be insufficient material
for all techniques, but for diagnosis, grading, and
staging, routine light microscopy will generally
be a priority.

Non-transitional tumours
In assessing biopsy or cystectomy specimens
with adenocarcinoma or squamous cell carcin-
oma, the same general features are looked for
and reported by the pathologist, and the
presence or absence of glandular metaplasia
and hyperkeratotic squamous metaplasia are
also highly relevant. In the case of adenocar-
cinoma the immunohistochemical absence of
prostate specific antigen or prostatic acid
phosphatase is helpful in excluding infiltration
by prostatic carcinoma. For rare types of
carcinoma (small, giant, or clear cell) the diag-
nosis itself is the principal prognostic factor,
insufficient numbers of cases being reported to
assess other factors. Categorisation of lymph-
omas will also require the application of
immunohistochemical techniques on formalin
fixed or frozen tissues.

In many instances of rare tumours the
opportunity for optimal investigation usually
arises from a rebiopsy or wider excision
subsequent to the initial diagnostic biopsy.
Most non-epithelial bladder tumours can be
diagnosed by light microscopy supported by
immunohistochemistry, and for spindle cell
lesions, the diagnosis of malignancy, although
at times extremely difficult, for assessing a
postoperative spindle cell nodule—can usually
only be made in this way. For subclassifying
sarcomas, electron microscopy may have a
role,14 particularly as more sensitive immuno-
histochemical techniques are reducing the
specificity of markers in this tumour category.
A small portion of tumour tissue should be placed
into a suitable fixative containing glutaralde-
hyde. Even if not eventually required for
diagnosis, it is good practice to collect samples
for electron microscopy in any case where the
diagnosis is difficult or unusual.

Non-neoplastic disease
Biopsy specimens taken during the investi-
gation of what proves to be a benign condition
are commonly performed in an attempt to
eliminate premalignant lesions from the
differential diagnosis. Thus in patients with
symptoms of bladder irritability biopsy
specimens are taken to exclude carcinoma in
situ, although morphological support for a
diagnosis of interstitial cystitis may become
evident. Similarly, in patients with chronically

Figure 7 Ejaculatory duct extending through the prostate from the seminal vesicle (upper right) to its orifice in the urethra (lower left). The latter is the site of the tumour (arrowed) (haematoxylin and eosin).
Gross examination of bladder specimens

inflamed bladders sampling excludes keratinising squamous metaplasia (important because of its malignant potential). At cystoscopically examination in the course of investigation of haematuria or recurrent infections, or before transurethral resection of prostate, biopsied abnormalities may include a variety of reactive lesions, only rarely showing specific features (tuberculosis and schistosomiasis), or amyloid. Biopsy specimens are described and processed as outlined above.

In the case of suspected interstitial cystitis it has been suggested that the presence of excess mast cells in the muscularis propria gives support to the clinical diagnosis. A more recent investigation, however, did not find any morphological feature to be pathognomonic of interstitial cystitis. It is not the authors’ experience that urologists request mast cell counts or that established treatment schedules exist which depend on such results. Should this investigation be required it is advised that laboratories establish their own normal range for mast cell counts per square millimetre of muscle.

2 Parmar MKB, Freedman LS, Hargrave TB, Tolley DA. Prognostic factors for recurrence and follow up policies in the treatment of superficial bladder cancer: report from the

M C Parkinson and C Fisher

doi: 10.1136/jcp.44.11.890

Updated information and services can be found at:
http://jcp.bmj.com/content/44/11/890.citation

**Email alerting service**

*These include:*
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/