Quantitative histopathology can aid diagnosis in painful bladder syndrome

R Thilagarajah, R O'N Witherow, M M Walker

Abstract

***Aims*—To define the pathology of painful bladder syndrome using a morphometric method.**

***Methods*—Bladder biopsy specimens from 31 patients with painful bladder syndrome and 11 controls were stained and examined at ×260 magnification with the aid of a 100 square counting grid. Random counts of the different tissues and inflammatory components were made to ascertain whether constant differences occurred that could be used to define the pathology of this uncommon condition.**

***Results*—In the lamina propria of painful bladder syndrome specimens, a significant increase was seen in the concentration of lymphocytes, T cells, and blood vessels; a decrease was seen in the number of fibroblasts, and no change was seen in the number of mast cells and macrophages. B cells were sporadic. The basement membrane in these specimens showed significant discontinuity and there was increased collagen deposition in the underlying muscle when compared with controls.**

***Conclusion*—Painful bladder syndrome exhibits constant histological features that may be used to aid diagnosis in this uncommon condition. Simple numerical cell/tissue measurement of this kind is also useful when treatment trials are considered, because objective statistical analysis (pretreatment and post-treatment) is possible without the need for expensive and complicated equipment.**

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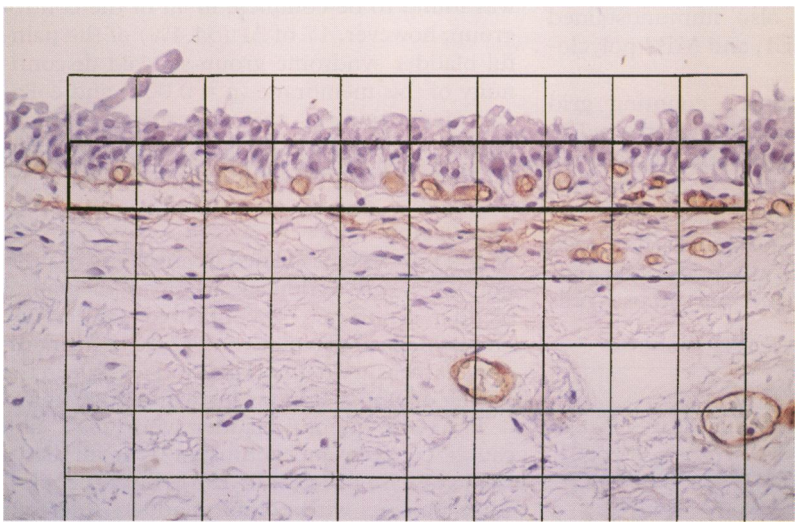

Figure 1 The grid is positioned as shown and the number of submucosal blood vessels in 1 × 10 squares is counted (Q-BEND10 stain; original magnification ×260).

Keywords: painful bladder syndrome; morphometry; diagnosis

Painful bladder syndrome is a clinical diagnosis comprising symptoms of frequency, suprapubic pain, and occasionally urgency, nocturia, and dysuria, persisting for a period of more than three months with sterile urine cultures and no loss of bladder capacity.[1] Patients with this condition are often diagnosed as having interstitial cystitis; however, this appears to be a separate entity with a different clinical course. Interstitial cystitis was first described by Hunner in 1914 as a syndrome with the above clinical features and characteristic ulceration of the bladder, resulting in fibrosis and loss of vesical capacity.[2] Previously, painful bladder syndrome has been described as "early" interstitial cystitis, lacking ulceration at cystoscopy[3 4]; however, there is a group of patients in which ulceration does not develop even after many years. Over a period of 10 years, we have investigated a group of patients at the urology unit of St Mary's Hospital with clinical symptoms of painful bladder syndrome, who showed no ulceration or loss of bladder capacity at cystoscopy.

A previous histological and immunofluorescence study carried out by Witherow *et al* showed an increase in chronic inflammatory infiltrate, mast cells, and new blood vessel formation in the submucosa. These were scored semiquantitatively according to site.[1] This type of scoring method is a subjective measure and, therefore, is of less use when detecting true statistical differences between cases.

Measurement of tissues and tissue components can involve the use of computerised image analysis systems. Although the accuracy of such methods is excellent, they remain elaborate and expensive research tools and are, as yet, not in routine use in most histopathology laboratories. Previously, microscope counting grids have been used in biological morphometery to measure tissue volume density,[4 5] and such a method has been modified here to determine whether significant histological features are consistent in painful bladder syndrome.

Materials and methods

Bladder biopsy specimens were examined from 44 patients (mean age 54, range 24–86), including 33 patients who presented with a clinical diagnosis of painful bladder syndrome supported histologically with a chronic inflammatory infiltrate and apparent submucosal angioneogenesis, and 11 normal controls selected from cystoscopy bladder biopsies

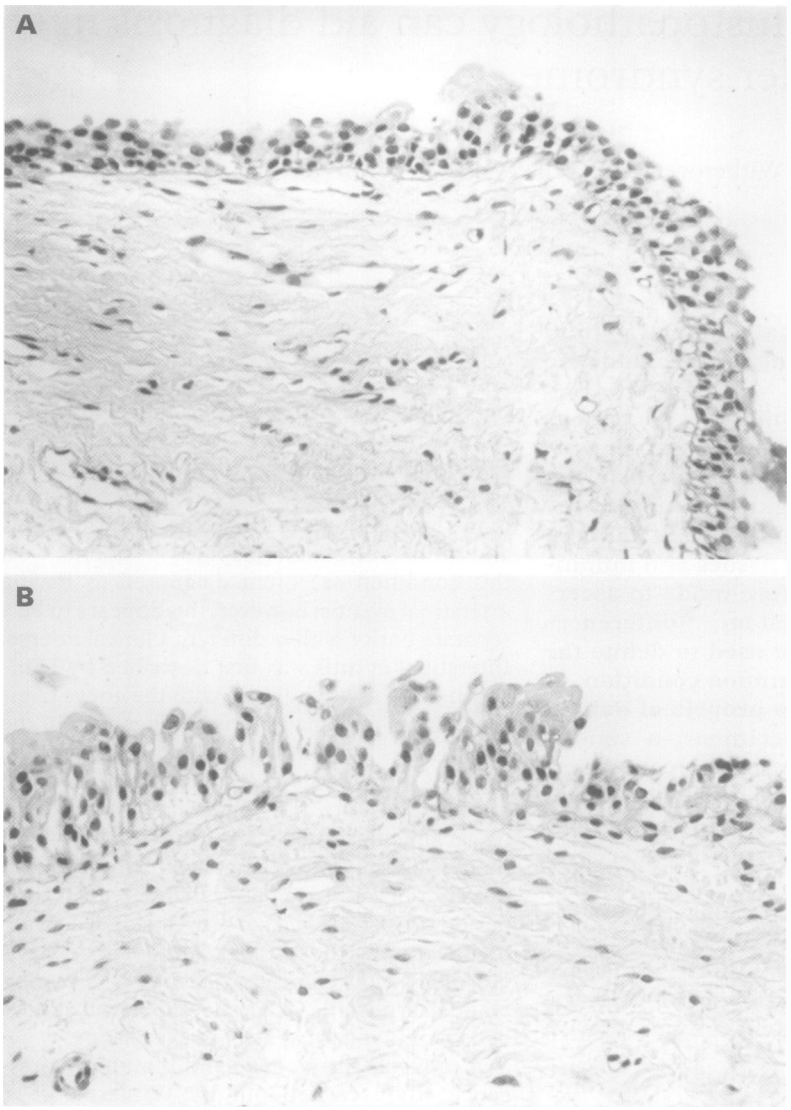

Figure 2 Light micrographs of bladder wall sections demonstrating continuous (A) and discontinuous (B) basement membranes (periodic acid Schiff's stain; original magnification ×200).

taken over a four year period from patients with upper tract pathology.[6]

Formalin fixed bladder biopsy specimens were routinely processed and paraffin wax sections cut at 3 μm were stained with haematoxylin and eosin, Gimenez,[7] toluidine blue, Elastin van Gieson, periodic acid Schiff's, alcian blue; they were also immunostained with Q-BEND10, UCHL1, and MB2 polyclonal antibodies.

A microscope 100 square counting grid (Leitz, Stuttgart, Germany) was then used at ×260 magnification to count the number of chronic inflammatory cells, T cells, B cells, macrophages, fibroblasts, and mast cells in the lamina propria of the specimens. For each count, the eyepiece grid was positioned randomly over the tissue and only those cells within complete grid squares were included. Random positioning was achieved by "defocusing" the specimen while looking through the eyepiece, moving the slide so that the grid lay over a section of the specimen and then bringing the image back into focus for tissue component measurement. This method provided an objective random selection at each count. This was repeated five times for each specimen, making sure that fields were not consecutive. To assess submucosal blood vessel concentration, the grid was randomly placed parallel to the mucosal surface and the number of blood vessels in a field comprising 1×10 grid squares was counted (fig 1). As before, this was repeated five times for each specimen to give a representative count. Finally, the specimens were examined for a continuous or discontinuous basement membrane (fig 2) and an increase or decrease of collagen in the underlying detrusor muscle (fig 3).

The results were statistically analysed using the Mann-Whitney U test and Fisher's exact probability test to ascertain whether there were histological differences between the two groups. Significance was taken at $p < 0.05$.

Table 1 Examination of cell components in painful bladder syndrome and controls

	Painful bladder syndrome (n = 31) (mean (SEM))	*Controls (n = 11) (mean (SEM))*	*p values*
Chronic inflammatory cells	32.5 (21.3)	10.1 (6.5)	< 0.0001
Blood vessels	7.8 (1.5)	4.4 (1.5)	< 0.001
T cells	27.1 (14.9)	6.7 (2.4)	< 0.0001
Macrophages	2.3 (1.4)	2.4 (1.1)	0.76
Mast cells	1.2 (1.6)	2.0 (1.4)	0.08
Fibroblasts	3.5 (1.1)	7.4 (1.8)	< 0.0001

Results

Of the original 44 biopsy specimens selected, two in the painful bladder syndrome group were found to contain numerous organisms when stained with the Gimenez stain. These were shown to be Gram negative rods and, therefore, the specimens were excluded from further examination.

The remaining 31 painful bladder syndrome and 11 normal control specimens were examined and the results outlined in table 1. There was a significant increase in chronic inflammatory cells, T cells ($p < 0.0001$), and submucosal blood vessels ($p < 0.001$) in the painful bladder syndrome group, with a reduction in the concentration of fibroblasts ($p < 0.0001$) in the lamina propria. The numbers of mast cells and macrophages were similar for both groups ($p = 0.08$ and $p = 0.76$, respectively). B cells in both groups were sparse and, therefore, these were excluded from the statistical analysis.

The integrity of the basement membrane was found to be complete in all of the control group; however, 17 of 31 (54.4%) of the painful bladder syndrome group showed discontinuity of this membrane ($p = 0.004$) and collagen deposition in the underlying detrusor muscle was significantly increased in the painful bladder syndrome group ($p = 0.004$).

Discussion

This study was performed to answer two questions: (1) can a clinical diagnosis of painful bladder syndrome be confirmed consistently on histological grounds? and (2) is tissue component measurement of this kind feasible in the setting of a busy urology/histopathology unit?

The term "painful bladder syndrome",[1] still causes concern and confusion as it implies a single disease process. In 1951, Bourque[8] classified patients with painful bladders into four categories: those with renal tuberculosis, those with Hunner's interstitial cystitis,[2] those with

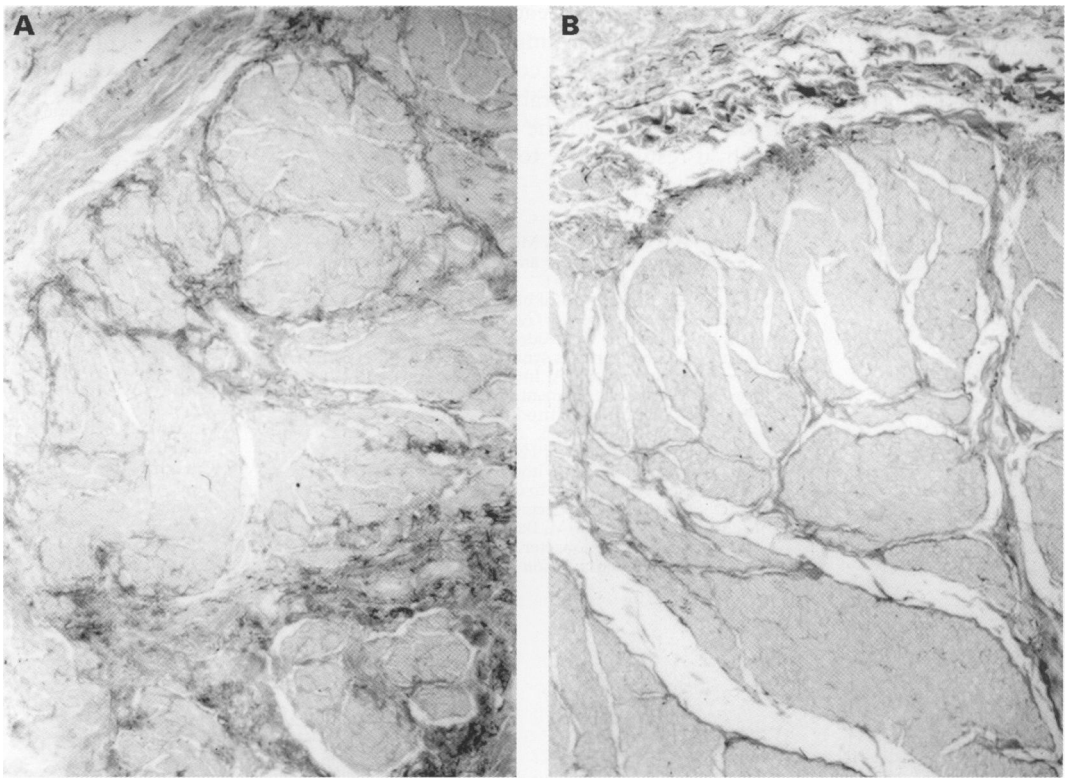

Figure 3 Collagen deposition in the underlying muscle in the bladder wall of a patient with painful bladder syndrome (A), compared with a normal control (B) (Van Gieson stain; original magnification ×200).

infective chronic cystitis, and finally those with inoperable bladder cancer. Today, the diagnosis of interstitial cystitis is one of exclusion, based on the criteria put forward by the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases in 1987.[9] The clinical picture of painful bladder syndrome has been described as early and grade 1 interstitial cystitis to account for the non-ulcerous nature of the condition[3 4 10 11]; however, we argue that with the clinical history, cystoscopic findings of normal vesical capacity and glomerulations after bladder distension, and characteristic histological findings, painful bladder syndrome is a specific diagnosis.

Our results demonstrate that a chronic inflammatory infiltrate is a constant feature and it appears to be an exclusively T cell mediated response, rather than a humoral immune response, as evidenced by the almost complete lack of B cells. Submucosal angiogenesis in the form of both small vessels and endothelial buds was present in abundance (fig 1). The reduction in the number of fibroblasts in the lamina propria is interesting, although it may be artefactual. Oedema of the tissue occurs in this condition and may cause dispersion of these "background cells". The influx of chronic inflammatory cells also leaves less physical room in a given field of tissue, effectively pushing the ground substance and cells of the lamina propria apart. Our finding of similar concentrations of mast cells in the lamina propria in both painful bladder syndrome and control specimens is in direct contrast to that of interstitial cystitis, where infiltration of the bladder wall is seen,[12 13] and again this implies a differing pathology.

Increased collagen deposition was present in the underlying muscle and probably represents a degree of fibrosis in response to the chronic inflammation. This does not lead to a reduction in the bladder capacity of these patients, but might cause a change in bladder sensation and, therefore, aggravate the symptoms of frequency. The finding of significant basement membrane discontinuity is relevant because damage may have been caused by an outside agent or the membrane may be inherently defective. This may be a similar mechanism to that seen in the protective mucosal glycosaminoglycan layer in certain conditions.[14-16] As with the glycosaminoglycan layer in patients with interstitial cystitis, the protection that the basement membrane offers to the underlying tissue is breached and may allow an as yet unidentified agent to penetrate and initiate the inflammatory reaction. As inflammation continues, further damage may occur and a vicious circle ensues. The glycosaminoglycan layer in the specimens was absent, which was attributed to the fixation process used and, therefore, the results were discounted.

The measurement method used in this study is accessible, cost effective and, once practised, not time consuming. In the setting of a busy urology/histopathology unit, this method may be advocated in cases of uncertainty. Many study groups use computer processed analysis for tissue component measurement as it gives consistent and accurate results.[12 17 18] The drawbacks of such systems include cost, availability, and the need for specific software that might hinder the inexperienced. In these days of ever decreasing research funding, methods such as the one demonstrated here should be welcomed. Detractors would argue that accuracy of results will suffer; however, we have shown that by having a specified number

of random counts for each specimen, spurious results are kept to a minimum.

The use of the grid counter in a pilot study will also add considerable weight to a future larger study without the initial financial outlay which, if results prove to be inconclusive, may be wasted.

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