# MAST CELLS AND NERVE FIBERS IN INTERSTITIAL CYSTITIS (IC): AN ALGORITHM FOR HISTOLOGIC DIAGNOSIS VIA QUANTITATIVE IMAGE ANALYSIS AND MORPHOMETRY (QIAM)

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## ABSTRACT

**Objective**. To develop and evaluate a diagnostic algorithm based on the alteration of mast cell and nerve fiber observed in bladder tissue of patients with interstitial cystitis (IC).
**Materials and Methods**. Non-IC samples from 6 control groups [N = 10. 10, 13, 2, 11, and 3, respectively) and nonclassic interstitial cystitis (NC-IC, N = 20) were stained with Giemsa stain in order to calculate the detrusor to mucosa mast cell ratio (DMMCR) using quantitative image analysis and morphometry [QIAM). Immunohistochemical staining for S-100 protein was also performed to quantify nerve fiber proliferation in the detrusor muscle of the bladder.
**Results**. The average DMMCR of NC-IC was 1.19, Bacille Calmette-Guerin (BCC) cystitis was 0.84 and microscopically normal bladder tissue from patients with bladder or prostate cancer was 0.45. No case of IC that we examined had a DMMCR <0.5. The number and percentage area of nerve fibers in the detrusor in IC were increased compared to controls and BCG [IC, 2.01%; BCG, 0.95%; control, 1.3%).
**Conclusion**. A diagnostic algorithm is proposed for IC based on the findings that indicate that: 1) if the DMMCR > 0.75, then IC is present; 2) if the DMMCR < 0.5. then IC is negative; and 3) if the DMMCR is between 0.5 and 0.75, a quantitative S-100 protein staining analysis can be employed to evaluate nerve fiber proliferation to detect those marginal cases of NC-IC. The findings of the study also suggest that a neuroimmune process or mediation may be involved in the pathogenesis of IC- UROLOGY 49(Suppl 5A): 41-47, 1997. 

Interstitial cystitis (IC) has been a diagnostic enigma, both clinically and histopathologically, since its first clinical description by Skene in 1887. He described a set of symptomatic features, later defined as cystitis parenchymatosa by Nitze in 1907.[1,2] The mucosal finding of a solitary ulcer that was distinguishable from infectious ulcers was first described by Hunner in 1914.[3] The presence of Hunner's ulcer on the bladder wall of patients with nonspecific, urinary symptoms of frequency, urgency, pelvic and suprapubic pain, and dyspareunia relieved variably by voiding became the standard diagnostic criteria of what is now considered classic (ulcerative) IC. However, this classic presentation occurs in <20% of patients.[4-7] The remaining 80% of patients exhibit the same constellation of symptoms but on cystoscopy show multiple, petechial hemorrhages ("strawberry-like") called glomerulations with no ulcer.[7] Biopsy of these bladders shows small mucosal fissures, a mild nonspecific chronic inflammatory infiltrate, suburothelial hemorrhages in the areas of glomerulations, edema, and vasodilatation in the submucosa and detrusor muscle and fibrosis. These nonspecific changes rule out most well-defined histopathologic entities but are not conclusive for IC and usually do not reflect the degree of cystoscopic findings or the severity of symptoms. Consequently, the diagnosis of IC is often based on clinical symptoms and cystoscopic findings without bladder biopsy. Interestingly, IC patients also suffer a higher incidence of other medical problems, such as allergies, irritable bowel syndrome, migraines, and some autoimmune diseases.[8]

There is no definite pathologic diagnosis of IC except for the presence of Hunner's ulcer. Histologic features seen on IC biopsies include edema, fibrosis, vascular ectasia, and nonspecific inflammatory cells, including mast cells,[7-9,12] Many researchers have claimed that mast cells are diagnostic of IC,[13-17] while others have shown that mast cell density is not predictive of IC.[7,9,11] We have shown in our previous work that a comparison of mast cell densities in the muscle and in the mucosa, that is, the detrusor to mucosa mast cell ratio (DMMCR), is predictive of IC.[18]

Recently, interest has turned to nerve fibers in IC. Hand, in 1949, described mast cells near nerve fiber bundles and an increase in nerve fibers.[19] In 1990, Christmas et al. showed evidence of nerve fiber proliferation in IC patients not seen in the control group.[20] Another investigation looked at mast cells and nerve fibers in ulcerative and non-ulcerative IC.[21] In the chronic ulcerative group, increases in nerve fibers correlated with increases in mast cells. Others have demonstrated that mast cells and nerve fibers in other species and organ systems have an intimate relationship and that nerve fiber innervation and function changes in response to inflammation.[22-24] Our lab has shown by electron microscopy a close spatial relationship between mast cells and nerve fibers in humans with IC and mice with experimental autoimmune cystitis (EAC) as well as evidence of cellular injury,[25] thus suggesting a neuroimmune mechanism for the pathology and symptoms of pain associated with IC.

In the present study, we continue to evaluate the diagnostic utility of the DMMCR and whether nerve fiber proliferation can be used as a diagnostic tool for IC when combined with the DMMCR. We have compared our control groups (autopsy specimens and normal microscopic bladder tissue from patients with cancer) to bladder biopsies from patients with cancer treated with chemotherapy, cancer treated with

radiation, Bacille Cal-mette-Guerin (BCG) cystitis, infectious cystitis, and IC. We will also introduce an algorithm for the diagnosis of IC, using the patient's clinical history, cystoscopy findings, DMMCR, and nerve fiber proliferation.

## MATERIALS AND METHODS

### PATIENT SELECTION

Patients with bladder biopsies or bladder resections were identified using the University of Pittsburgh Medical Center

Medical Archival Research System (MARS). Bladder biopsies from 20 IC patients were selected who fulfilled the National Institutes of Health (NIH) criteria for IC.[26] In brief, NIH criteria state that, to be included as IC, patients must have either glomerulations on cystoscopic examination or a classic Hunner's ulcer, and they must have either pain associated with the bladder or urinary urgency. Examination for glomerulations should occur after distention of the bladder under anesthesia to 80 to 100 cm of water pressure for 1 to 2 min. The glomerulations must be diffuse—present in at least 3 quadrants of the bladder—and there must be at least 10 glomerulations per quadrant. Of these 20 biopsies, 1 patient had ulcerative IC and 19 patients had nonulcerative IC. The control groups consisted of grossly and microscopically normal bladder sections from 5 autopsies and from uninvolved areas in 10 patients with either bladder or prostate carcinoma. Additional biopsies for comparison came from 11 patients treated with chemotherapy for either bladder or prostate carcinoma, 3 patients treated with radiation for bladder or prostate carcinoma, 13 patients treated with BCG for bladder carcinoma, and 2 patients with infectious cystitis. All biopsy specimens contained both bladder mucosa and detrusor muscle.

### DETERMINATION OF THE DMMCR

The detrusor to mucosa mast cell ratio (DMMCR) was determined by quantitative image analysis and morphometry (QIAM) as described in Hofmeister et al.[18] Serial tissue sections were stained with hematoxylin and eosin (Fig. 1) and with a standard Giemsa stain (Fig. 2). Previous work in this laboratory has shown that the different protocols for mast cell staining resulted in significant differences in the final calculated DMMCR. The choice of the Giemsa staining method was based on the ease of performing the stain and the relatively low cost of the stain. The areas occupied by the mucosa with submucosa and by the detrusor muscle of each bladder specimen were determined by first tracing the area of the entire specimen, then tracing the detrusor muscle area. The area of the mucosa with submucosa was derived by subtracting the detrusor area from the entire area:

$$\text{Area (mucosa)} = \text{Area (specimen)} — \text{Area (detrusor)}.$$

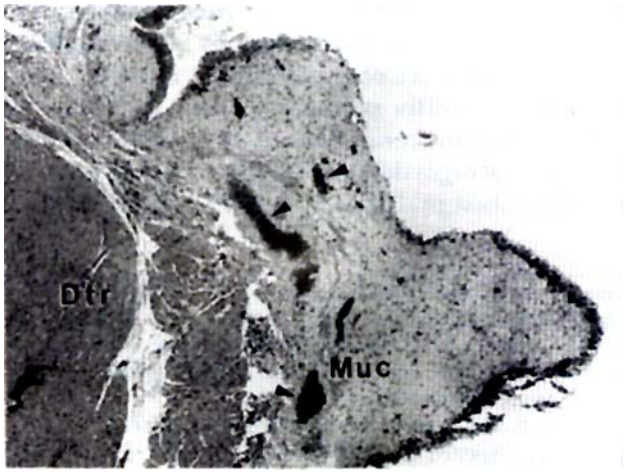


FIGURE l. *Cross-section of bladder wall from a patient with interstitial cystitis (hemotoxylin and eosin stain). The mucosa layer (Muc) shows typical injuries of 1C, including vasodilation (arrowheads), fibrosis, etc. Dtr: detrusor layer of the bladder wall.*

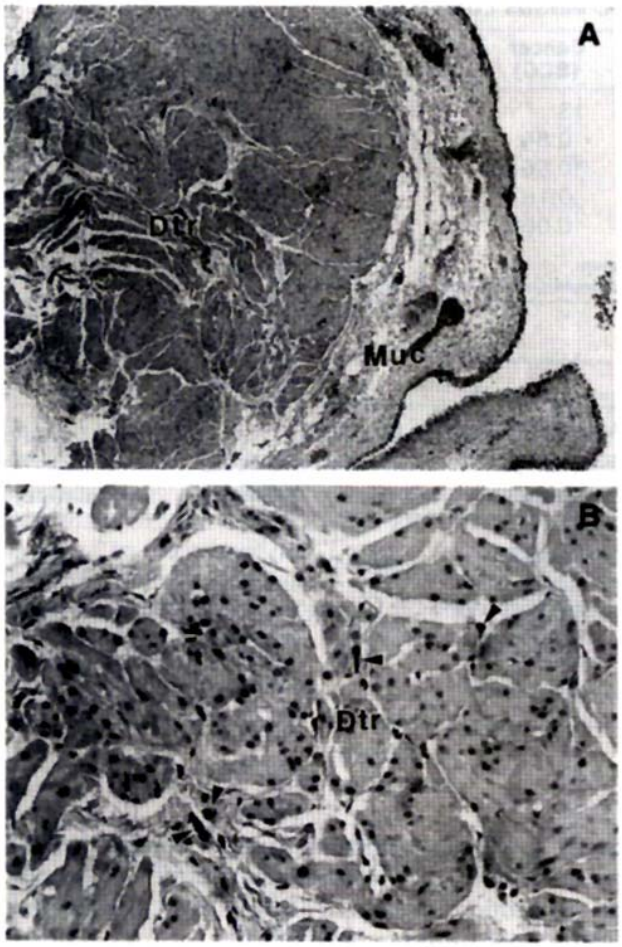


FIGURE 2. *Giemsa-stained bladder tissue of interstitial cystitis patient. (A) a low-power cross-section of bladder wall. Muc: mucosa. Dtr: detrusor. (B) a high-power view of the detrusor. Mast cells can be easily identified (arrowheads).*

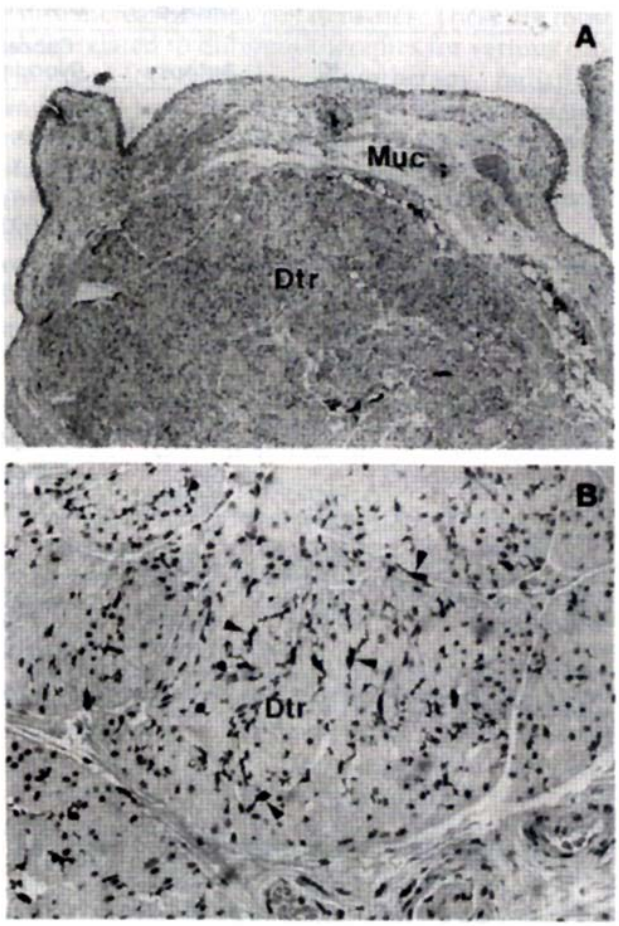


FIGURE 3. *SWO immunochemically stained bladder tissue of interstitial cystitis patient. (A) a low-power cross-section of bladder wall. Muc: mucosa. Dtr: detrusor. Most of the black dots seen were positively stained nerve fibers. (B) a high-power view of the detrusor. Arrowheads indicate S 100-positive nerve fibers.*

Mast cells were identified and counted in each area. The counts were stored and the mast cell densities were calculated as follows:

$$\text{Density of mast cells} = \frac{\text{Number of mast cells}}{\text{Area}}$$

The DMMCR was calculated by dividing the detrusor mast cell density by the mucosa mast cell density:

$$\text{DMMCR} = \frac{\text{Density of mast cells in the detrusor muscle}}{\text{Density of mast cells in the mucosa}}$$

DETERMINATION OF NERVE FIBER PROLIFERATION

Nerve fibers in each biopsy were identified by immunohistochemical staining for S-100 protein (Fig. 3). Quantitative image analysis and morphometry (QIAM) was used to determine the total nerve area within the detrusor muscle of each biopsy specimen.

The percentage of the nerve fibers was calculated by dividing the area of the nerve fibers by the

area of the detrusor muscle:

$$\text{Percentage or nerve libers} = \frac{\text{Area of nerve fibers X 100}}{\text{Area or detrusor muscle}}$$

Usually, sufficient detrusor muscle was present so that 5 areas of muscle from each specimen were evaluated for a total of approximately 0.230 $mm^2$, and the results were averaged.

DATA ANALYSIS

The DMMCR and nerve fiber proliferation parameters were determined for each patient. The averages were calculated for each group. Results were compared using the Student's t test.

## RESULTS

DMMCR

The DMMCR data is compiled in Table I and graphically represented in Figure 4. The DMMCR for the IC patients was 1.19 ± 0.15, which was significantly different from both the autopsy group, where the DMMCR - 0.67 ± 0.12 ($P < 0.05$), and the uninvolved bladder sections from cystectomies, where the DMMCR = 0.45 ± 0.13 ($P < 0.005$). The highest DMMCR (2.34 ± 0.79) was in the cancer treated with chemotherapy group and was also significantly different from that of the IC patients ($P < 0.05$). The DMMCR of the biopsy samples from the bladders of those treated with BCG therapy was 0.84, which fell just outside the parameters for a statistically significant difference. The DMMCR for the biopsy samples from irradiated bladders (1.34) and for those with infectious cystitis (1.72) also had a DMMCR greater than the IC group. However, our initial samples were too small for valid statistical comparisons.

**TABLE I. DMMCR: IC versus controls**

| | IC | Autopsy | Cancer (Normal) | Cancer (BCG) | Infection | Cancer (Chemo) | Cancer (Radiation) |
|---|---|---|---|---|---|---|---|
| Number | 20 | 10 | 10 | 13 | 2 | 11 | 3 |
| Mean | 1.19 | 0.67 | 0.45 | 0.84 | 1.72 | 2.34 | 1.34 |
| Standard deviation | 0.67 | 0.38 | 0-43 | 0.56 | | 2.62 | |
| Standard error | 0.15 | 0.12 | 0.14 | 0.16 | | 0.79 | |
| P* | | 0.016 | 0.002 | 0.065 | | 0.035 | |

** Student's t test, The value was obtained by comparing IC group with other individual groups.*
*DMMCR - detrusor to mucosa mast cell ratio; IC = interstitial cystitis; BCG = hadlle Calmette-Guerin; Chemo = chemotherapy.*

**TABLE II. Nerve area/total area: IC versus controls**

| | IC | Autopsy | Cancer (Normal) | Cancer (BCG) | Infection | Cancer (Chemo) | Cancer (Radiation) |
|---|---|---|---|---|---|---|---|
| Number | 20 | 10 | 10 | 13 | 2 | 11 | 3 |
| Mean | 2.01% | 1.05% | 1.31% | 0,95% | 0.04% | 1.41% | 1.24% |
| Standard deviation | 0.89% | 0.09% | 0.38% | 0.65% | | 0.39% | |
| Standard error | 0.20% | 0.03% | 0.12% | 0.18% | | 0.12% | |
| P* | | 0.001 1 | 0.0120 | 0.0004 | | 0.0215 | |

** Student's t test. The value was obtained by comparing IC group with other individual groups.*
*For abbreviations, see Table I.*

NERVE FIBER PROLIFERATION

The data of the nerve fiber area as a percentage of the total detrusor area is compiled in Table II and graphically represented in Figure 5. The bladder biopsy samples from the IC group had the highest average percentage at 2.0% ± 0.2%, which is significantly greater than all other groups tested.

## COMMENT

Many investigators have looked at various possible etiologies, (eg, infection, disruption of the epithelial glycosaminoglycan layer, and autoimmune mechanisms) to determine the pathogenesis of IC. In addition, they have also attempted to establish a set of criteria for the diagnosis of this disease. A definitive diagnosis of IC is based solely on the presence of Runner's ulcer seen on cystoscopy (classic IC), which represents <20% of all IC cases.[4-7] The diagnosis of nonclassic IC is currently based on a constellation of clinical symptoms without attributable cause after thorough history, microbiologic culture, and nonspecific cystoscopic findings have eliminated other forms of cystitis. In 1987, the National Institute of Arthritis,

Diabetes, Digestive and Kidney Diseases adopted an exclusionary set of criteria to be used for patients in IC research studies.[26] However, no clearly-defined set of criteria exists for the diagnosis of IC.

Mast cells have been postulated as having a central role in the pathogenesis of IC, especially by those who have identified increased numbers of mast cells within the detrusor muscle from a bladder biopsy. We have shown in previous work that the number of mast cells visualized is dependent on the type of staining procedure used for mast cells.[18] This conclusion is further supported by several investigators who have shown that the fixation method, the type of stain, and the staining methodology affects the number of mast cells visualized in paraffin-embedded bladder biopsies.[11,27] It is still controversial whether the increased number of mast cells can be used for diagnosis of IC. We believe that the reason for the controversy is that the methodology, such as fixatives, staining protocols, and conditions, etc, employed in the past were inconsistent in comparison to one another, which resulted in quite different absolute counts of mast cells. In our study, a detrusor to mucosa mast cell ratio (DMMCR) was adopted to quantify mast cells. It eliminates the possible bias of mast cell counts due to variable conditions when the tissue was processed.

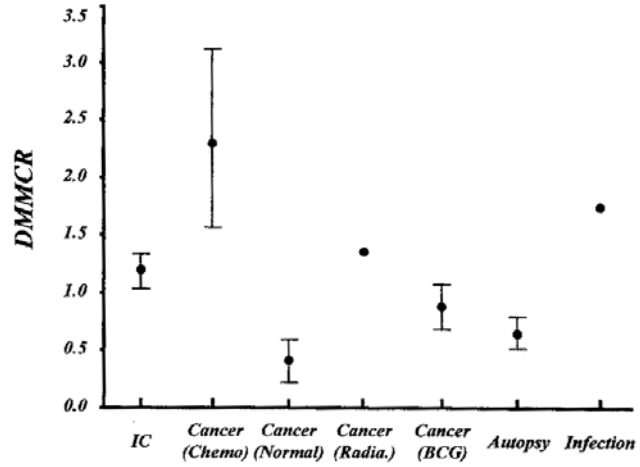


FIGURE 4. *Comparison of detrusor to mucosa mast cell ratio (DMMCR) between interstitial cystitis (IC) and control groups. DMMCR of IC was significantly higher than the autopsy and cancer (normal) groups and was marginal to the cancer (BCG) group. The cancer (chemotherapy) group had highest DMMCR value (see discussion for explanation). Cancer (radiation) and infection groups could not be compared due to limited samples.*

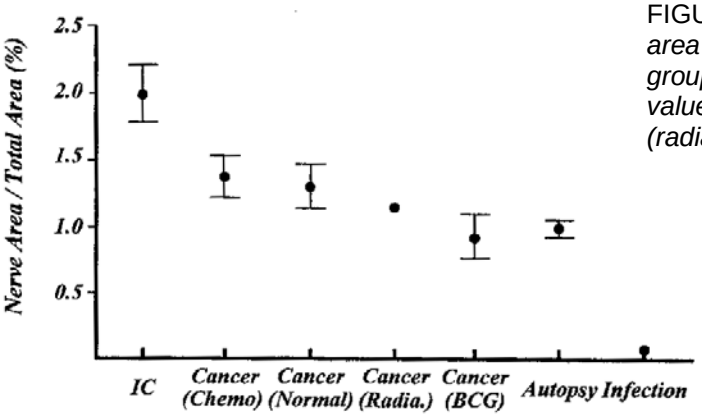


FIGURE 5. *Comparison of percentage of nerve area between interstitial cystitis (IC) and control groups. The IC group had significantly higher values than the controls except for the cancer (radiation) and infection groups.*

One problem with comparing mast cell counts is that the normal density of mast cells has not been established for the human bladder. A major obstacle has been the lack of adequate specimens suitable for a normal control sample. In this study, bladder tissue obtained from either biopsy specimens that were diagnosed with no significant pathologic change or sections that were grossly and microscopically uninvolved from untreated cystectomy specimens for bladder or prostate cancer were used. Only 2 adequate specimens diagnosed with infectious cystitis were obtained.

Our data show that other circumstances can cause increased mast cell densities. These are most likely related to different therapies for various carcinomas, for example, chemotherapy, radiation, and BCG. Sant and Theoharides have previously recognized that the numbers of mast cells in transitional cell carcinoma are increased, but adds that they are not activated as in IC.[8] However, by following our proposed criteria, these patients would not be investigated for IC as other causes for their symptoms would be known. These patients should also not be used as any control group in experimental studies for the diagnosis of IC.

The bladder biopsies from the carcinoma group with no treatment were mostly random biopsy specimens taken at follow-up and had DMMCR < 0.5. The biopsies from the autopsy group show DMMCR ranging from 0 to 1-14. However, the number of mast cells visualized in both the mucosa and muscle was low, and the corresponding mast cell densities were about 4 to 8 times less than the other groups.

Our initial investigations into the role of nerve fiber proliferation in IC bladders demonstrated ultrastructurally an intimate relationship between mast cells and nerve fiber endings, and also demonstrated that the nerve fiber endings near mast cells show signs of destruction.[25] Other studies of different organ

systems and tissues in both humans and animals have also shown relationships between mast cells and nerve fibers. These findings suggest that a common, neuroendocrine feedback mechanism exists between mast cells and nerve fibers.[22,24,28,31]

Other studies have shown that the numbers of nerve fibers in IC bladders are increased.[20,21] Using QIAM, we compared nerve fiber proliferation in IC and control groups. We were able to quantify the increased area of nerve fibers and determined a percentage of the area of stained nerve tissue for a given bladder muscle area. Bladder biopsies from IC patients had the highest percentage of nerve tissue at 2.01%. Other diagnostic groups had a value of approximately 1%. A higher DMMCR means that there were more mast cell counts in the detrusor. In IC cases, the results not only showed higher DMMCR (higher mast cell counts), but also increased nerve fiber area. This suggests that the 2 events were correlated. On the other hand, such a relation seemed not to be apparent among the control groups. This was especially obvious in the infectious cystitis group, which had a high DMMCR but a very low nerve fiber area percentage.

One of the hallmarks of IC is the pain experienced by the patients. Many methods have been used to try to curtail the pain and increase bladder capacity, including bladder hydrodistention and instillation of DMSO. This also continues to be an area of research as other methods are tried in an effort to reduce the painful and debilitating symptoms. Although many therapies partially alleviate the symptoms, none provide significant long-term relief. This symptomatology has prompted interest in the study of nerve fiber proliferation in IC bladders. Interactions occurring between mast cells and nerve fibers that are distinct from other pathologic mechanisms could be responsible for the clinical symptoms of pain, frequency, and urgency. A neurohormonal mechanism may be involved in which, after an initial triggering event, the mast cell contents instigate nerve fiber destruction and proliferation, and nerve fibers stimulate mast cells to degranulate, forming a positive feedback loop. This chain of self-reinforcing events may result in the typical, nondiagnostic, morphologic features of edema, fibrosis, vascular ectasia, increased numbers of mast cells, especially degranulated mast cells, and increased numbers of nerve fibers identified in a bladder biopsy.

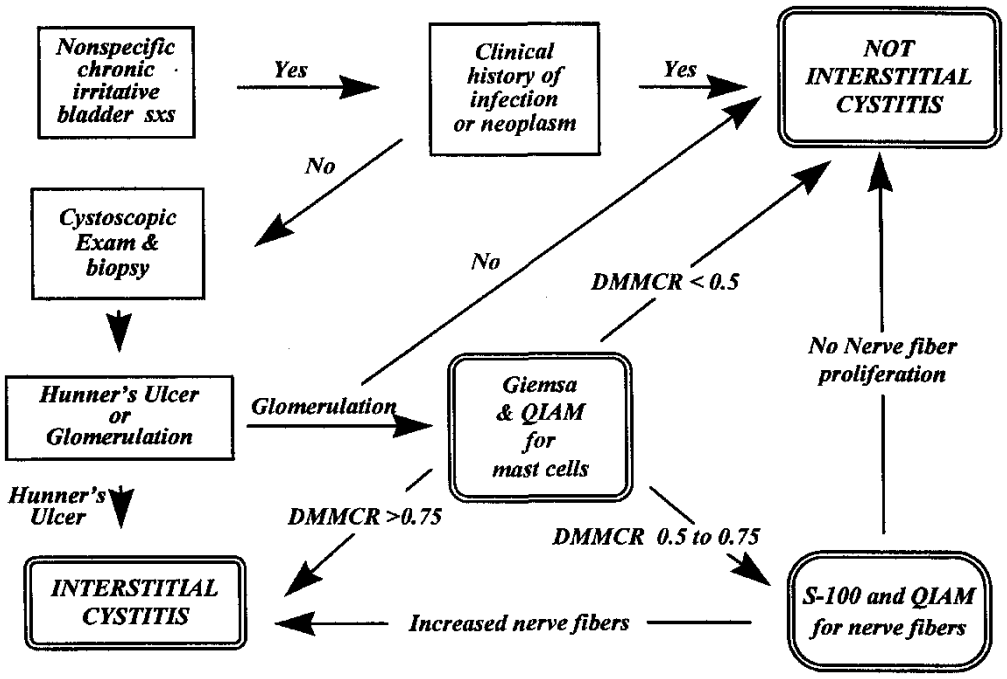


FIGURE 6. *A proposed diagnostic algorithm for interstitial cystitis.*

ALGORITHM FOR THE DIAGNOSIS

Based on these observations, we propose an algorithm for the diagnosis of interstitial cystitis (Figure 6). If a patient presents with irritative bladder symptoms for > 6 months that are not relieved by antibiotics, and cystoscopic exam rules out neoplasia or any other reason for these symptoms, but a Hunner's ulcer is observed, the diagnosis is classic interstitial cystitis. If only glomerulations are seen under cystoscopy, a biopsy that includes both mucosa and detrusor muscles should be obtained. A routine hemotoxylin and eosin stain as well as a Giemsa stain should be initially performed. Then a quantitative analysis of DMMCR can be computed. If the DMMCR > 0.75, a diagnosis of nonclassic IC is more likely. If the DMMCR < 0.5, IC is less likely. For a DMMCR between 0.50 and 0.75, the biopsy should then be immunohistochemically stained for S100 protein. If the percentage of nerve fiber area is increased, a diagnosis of nonclassic IC is more likely.

The use of the algorithm for clinical diagnosis of IC has raised inquiries of how practical it will be, as the diagnostic processes involve certain special tissue stains, computer-aided image analysis, and additional processing time. We believe that it is unnecessary to have classic IC biopsy samples proceed with the procedure, unless the procedure's purpose is for research rather than clinical diagnosis. But for prospective nonclassic IC biopsies, which represent 80% of total IC cases according to previous investigations,[7] our study provides an alternative way to quantify the cellular changes in the tissue and facilitate the final diagnosis of IC.

## CONCLUSIONS

We have shown that mast cells and nerve fibers play an important role in the diagnosis of interstitial cystitis. This was a retrospective study, and future plans include using the algorithm in a prospective study. The relationship between the nerve fibers and mast cells in IC may provide additional avenues of research into possible etiologies and pathogenic mechanisms of IC. From such studies, new treatments may be targeted not only at the symptoms but at the cause.

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