# 114A

**Authors:** A Rosamilia , L Cann, P Dwyer, P Rogers
**Institution:** Monash Medical Centre, Royal Women's Hospital
**Title:** BLADDER SUBMUCOSA STEROID RECEPTORS IN WOMEN WITH INTERSTITIAL CYSTITIS , FEMALE AND MALE CONTROLS

**Aims of Study**

Interstitial cystitis predominantly affects women rather than men with a ratio of 10:1. The aim of this study was to quantitatively assess any difference in oestrogen and progesterone receptor expression in the submucosa of bladder biopsies in a cohort of women with IC and control males and females subjects.

**Methods**

Following standardised bladder hydrodistention, biopsies were obtained from 26 IC and 28 female control subjects. Bladder biopsies were obtained from the area with maximum glomerulations in the IC subject and usually from the posterior or lateral bladder walls in the control subject. There were no biopsies in either control or IC subject from the trigonal area. In the male control group biopsy material was archival tissue from 5 men having cystectomy for bladder carcinoma. A streptavidin biotin immunohistochemical protocol was used with monoclonal anti-oestrogen receptor (ER) antibody and anti-progesterone receptor (PR) antibody. A dual stain technique with PR and Actin, alpha smooth muscle antibody was employed to identify the cell type. The immunostaining was assessed semi-quantitatively using a rectangular sampling window of size 0.02mm$^2$. Four scores were obtained for each section at a high intensity setting of the image analyser and four scores at a low intensity setting. The high intensity score was the count of the very intensely stained cells only whereas the low intensity score was the count of all the stained cells including the intensely stained cells. The results were analysed using the Mann-Whitney test.

**Results**

ER and PR immunohistochemistry was performed on 26 IC subjects, 26 female control subjects and 5 control ma subjects. There was no significant difference in ER positive submucosal cells in the bladder biopsies of control women or women with IC at the low intensity setting ($p=0.58$) but a trend toward increased number of cells in the control group at high intensity settings ($p=0.08$). There was no difference in ER positive submucosal cells in the bladder biopsies of control females or males at both low ($p=0.14$) and high ($p=0.28$) intensity settings. The control males had a greater number of ER positive submucosal cells compared with the IC group at both low ($p=0.04$) and high ($p=0.08$) intensity settings.There was no difference in submucosal cells staining positively for PR between female control and IC subjects at low intensity ($p=0.13$) or high intensity ($p=0.6$) settings. There was a significantly increased number of PR submucosal cells in bladder biopsies derived from male subjects as compared with eithe female controls ($p<0.01$) or female IC subjects ($p<0.01$).The dual stain immunohistochemical study with antibodies to PR and smooth muscle actin had an appearance consistent with a stromal smooth muscle cell showing progesterone staining.

**Conclusion**

This is the first study of bladder submucosal oestrogen and progesterone receptors in IC apart from a small study which dealt with the relationship between steroid receptors and mast cells (1). The current study demonstrated no significant difference in the number of submucosal cells staining positively for ER or PR between IC and female controls. ER positive cells were present in about half of the control and IC subjects but

generally with weak to moderate staining. Eighty per cent of the IC and 50 % of the control group demonstrated bladder submucosal cells positive for PR with 14 % of both control and IC groups showing intense staining cells.

Male controls showed a trend toward a greater number of ER positive cells and a significantly greater number of PR positive cells compared with female controls or those with IC.

In the current study, there is no evidence of a difference in the number of oestrogen (ERα) or progesterone (PR) receptor staining submucosal cells in bladder biopsies from women with IC as compared with controls. The analysis was only of submucosal cells and did not study the urothelial or deeper detrusor muscle layers.The higher level of progesterone staining cells in the male bladder was interesting and may be due to a low circulating progesterone level; may have been stimulated by the concurrent bladder carcinoma.or due to the difference between tissue obtained at cystectomy versus after hydrodistention. It in fact may reflect a real male/female difference for example with males having a greater submucosal cellularity with consequently more of these stromal cells positive for progesterone. These stromal cells are smooth muscle cells which stain positive for actin.

# 114B

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**Institution:** Monash Medical Centre, Royal Womens'Hospital
**Title:** BRADYKININ SUBTYPE 1 RECEPTOR IMMUNOHISTOCHEMISTRY IN INTERSTITIAL CYSTITIS

**Aims of Study;**

An increase in the urinary levels of the bradykinin-(1-8) peptide in women with interstitial cystitis (IC) has previously been described (1). This peptide is known to act solely on the bradykinin subtype 1 (B1) receptor which has been shown to be induced or up regulated in animal models of inflammation. In this study the aim was to

confirm B1 receptor expression and document its localisation in bladder biopsies from female controls and to determine whether it is increased in bladder biopsies of women with IC

identify the cell population demonstrating bradykinin-1 receptor positivity with the use of double stain immunohistochemistry

**Methods:**

A streptavidin biotin immunohistochemical protocol was used with antigen retrieval. The slides were assessed and scored in a blinded manner so that the identity and diagnosis was not known. The scoring was semi-quantitative and ranged from a minimum of 0 (no staining) and a maximum of 3 (maximal staining). A preabsortpion experiment was performed. Dual immunohistochemistry with antibodies to BK1 and the populations of B lymphocyte, macrophage, mast cell and T lymphocytes was performed. Statistical analysis was by the Mann-Whitney test.

**Results:**

Bladder biopsies from 27 subjects with IC and 26 control subjects were assessed. There was a trend toward a greater number of biopsies with bradykinin 1 receptor staining in the IC as compared with the control group ($p=0.09$). Close inspection of the staining suggested cytoplasmic staining in stromal cells (possibly superficial fibroblasts), inflammatory cells (possibly lymphocytes) and to a variable degree in epithelial cells. The endothelium, the deep submucosal cells, detrusor mast cells and erythrocytes were consistently negative.

The BK1/CD20 double stain showed some cells staining positive for both BK1 and CD20 (a pan B cell marker. However the BK1 staining cells appeared distinct from cells staining positive for the macrophage and neutrophil. There may have been some mast cells which also stained for BK1 receptor but the majority were differently staining cells, and the T lymphocytes were a distinct population also.

**Conclusion:**

This study showed a trend toward increased numbers of cells demonstrating positive staining for bradykinin-1

receptor (p=0.09) in the bladder submucosa of subjects with IC as compared with female control subjects. It is consistent with the only other bradykinin 1 receptor immunohistochemical study performed in IC which reported expression of bradykinin 1 receptor subtype in 9 of 20 IC bladder biopsies but none of the 7 cadaveric bladder or 4 human aorta specimens (2). The double stain immunohistochemistry study suggests that some of the positive BK1 cells could be a subpopulation of B cells. Further dual immunohistochemistry with smooth muscle actin is planned. Recently immunohistochemical evidence for B1 receptor expression in the rat sensory nervous system has been reported, specifically in the dorsal horn of the spinal cord where primary afferents terminate, and in peripheral nerve terminals in the bladder (3). This distribution of B1 receptors in sensory neural pathways suggests a role in hyperalgesia.

There are limitations of the current study. There are the documented technical problems with the polyclonal antibody, its inconsistency and apparent lack of specificity. In addition the scoring is semi-quantitative and there are the problems of possible sampling error in drawing general conclusions from a small biopsy.

Nevertheless, it is possible that the BK1 receptor staining observed in this study is real as the findings are consistent with the previous study (2). Clearly, further experiments are warranted to definitively substantiate the involvement of the BK1 receptor in IC. If confirmed, the finding of induced bradykinin 1 receptor cells in IC would provide further evidence for an inflammatory pathogenesis, and a role for kinins in the bladder and the nervous system in producing the symptoms of pain, urgency, frequency and hyperalgesia.

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3) Wotherspoon G, Winter J. Bradykinin BI receptor is constitutively expressed in the rat sensory nervous system. Neurosi Lett 2000; 294: 175-8