Hypothesis / aims of study
The cellular localization and role(s) of different muscarinic receptors in human urinary bladder syndromes is uncertain. We have studied the muscarinic receptor subtypes M2 and M3 in the human urinary bladder, and related changes in the receptor density of patients with detrusor overactivity, painful bladder syndromes and controls to clinical measures such as urinary frequency and urgency.

Study design, materials and methods
Bladder specimens obtained from patients with painful bladder syndrome (PBS, n=11), idiopathic detrusor overactivity (IDO, n=12), and asymptomatic microscopic hematuria (controls, n=16), were immunostained using specific antibodies to muscarinic receptor subtypes M2, M3 and vimentin (a marker for myofibroblasts). Results of immunostaining were quantified with computerized image analysis, and were correlated with the clinical dysfunction (Frequency and Urgency scores).

Results
M2- and M3-immunoreactivity was observed in the urothelium, nerve fibres, and detrusor layers: in addition, strong "myofibroblast-like" cell staining, similar to vimentin, was present in the suburothelial region and detrusor muscle. A significant increase in the sub-urothelial myofibroblast-like M2-immunoreactivity was seen in both PBS (P=0.0062) and IDO (P=0.0002), and myofibroblast-like M3-immunoreactivity in IDO (P=0.0122), with a trend in PBS. The M2- and M3-immunoreactivity significantly correlated with the Urgency score (P=0.0002 and 0.0206 respectively) (fig c and d), and M2-immunoreactivity with the Frequency Score (P=0.0029)(fig a). No significant difference was seen in the M2-, M3 -urothelial and detrusor or vimentin- immunostaining.

Fig (1a) frequency score compared with M2 immunoreactive area (1b) M3 immunoreactive area

Fig (1c ) urgency score correlated against M2 immunoreactive area (1d) against M3 immunoreactive area
Bar charts showing the relative % area (mean ±SEM) of (2a) M2-immunoreactive myofibroblasts, (2b) M3- immunoreactive myofibroblasts, (2c) M2 / M3 Ratio (%) and (2d) Vimentin staining in control (n=16), IDO (n=12) and PBS (n=11) groups.

Interpretation of results:
This study demonstrates the cellular localisation and distribution of muscarinic receptors M2 and M3 in the human urinary bladder disorders. The increase in M2- and M3-immunostaining in myofibroblast-like cells in clinical bladder syndromes, and correlation with clinical scores, suggests a potential role in pathophysiological mechanisms and the therapeutic effect of anti-muscarinic agents.

Concluding message:
Muscarinic receptors do correlate with clinical symptoms in detrusor overactivity and painful bladder syndrome. M2 and M3 immunoreactivity correlate with urinary frequency and only M2 significantly correlate to urgency.

FUNDING: NONE
DISCLOSURES: NONE
HUMAN SUBJECTS: This study was approved by the Hammersmith Ethics Committee and followed the Declaration of Helsinki. Informed consent was obtained from the patients.