Reviewer's report

Title: A comparative study of cytokeratin 20 immunostaining in the urothelium of (1) normally-innervated bladder with transitional cell carcinoma, (2) normally-innervated bladder of individuals with cystitis, and (3) neuropathic bladder of patients with spinal cord injury

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Reviewer: Prof Jenny Southgate

Level of interest: A paper whose findings are important to those with closely related research interests

Advice on publication: Unable to decide on acceptance or rejection until the authors have responded to the compulsory revisions

Overview
1. The stated aims of this study were to investigate whether spinal cord injury affects terminal differentiation in the urothelium of the neuropathic bladder, based on the expression of cytokeratin 20 by superficial umbrella cells. Although this raises the intriguing question as to whether aspects of urothelial maturation may be dependent upon normal bladder function, this has not been given full consideration in the current manuscript. Given the stated aims, the reasons for inclusion of a limited series of specimens from individuals with cystitis and transitional cell carcinoma are unclear, as both disease groups are extremely diverse and heterogeneous, and hence, cannot be adequately represented by such a small number of specimens. I suggest that group 1 and group 2 patients are omitted from this study to make it more focused (Compulsory)

2. It is a shame that the authors only investigated cytokeratin 20 expression and did not broaden their study of the neuropathic urothelium to include a range of other epithelial markers, such as other cytokeratins and uroplakins, that would have a) verified objectively that the epithelium was transitional and did not show any underlying squamous or other metaplastic change undetected by histological analysis and b) whether there was a general failure to undergo terminal differentiation or just specific loss of cytokeratin 20 expression. This could have led to a more informed discussion of the potential mechanisms. (Discretionary)

Specific points (all Compulsory)

Abstract
3. The term cystitis should be broadened to "bacterial and non-bacterial cystitis".
4. It is unnecessary in the Abstract to mention the percentage of formaldehyde used.

Introduction
5. It is speculation that secreted urinary proteins play a crucial role in regulating desquamation of urothelium.
6. Reference 2 refers to rat urothelium and it would be more appropriate to substitute a reference referring to CK20 expression in human urothelium, of which there are many.
7. The authors should include Scriven et al (J Urol 1997; 158: 1147-1152) with references 3 & 4.
8. There is a considerable literature on the expression and significance of cytokeratin 20 in urothelial cancers that has been ignored. If the data on TCC is included, then this literature should be referred to in the Introduction or Discussion (although I feel it should be omitted - see above).

Patients and Methods
8. What is Paraplast?
9. Avoid use of "etc" in the description of therapeutic procedures.
10. The sentences "All were given a copy of the patient information sheet..... A medical practitioner...... I the patients were willing to participate in this study ...." should be omitted as unnecessary.
11. Paragraph 3 - repeat of how biopsies were fixed and embedded.
12. Were histological features reported by a trained histopathologist? What do the authors mean by none of the 63 biopsies from spinal cord injury patient showed "definite evidence" of dysplasia or neoplasia. State what criteria were used to identify umbrella cells. This is important as, without more objective evidence, the rest of the paper rests on the histological identification of transitional differentiation.
13. The antibody used in the study should be referred to by its clone name, Ks20.8, rather than the Novocastra catalogue name.
14. What is meant by a "standard ABC technique" - do the authors mean immunoperoxidase and was a kit purchased from a company and if so, from where?

Discussion
15. The authors say that in a previous study, only 18 or 37 biopsies from neuropathic bladders were positive to secretory IgA. Were any of the same biopsies used in this study, and if so, was there any correlation between cytokeratin 20 and sIgA expression or loss?
16. The discussion, as written, is inconcise and would benefit from being rewritten. The authors appear to suggest that absence of cytokeratin 20 could help a histopathologist distinguish between benign and malignant changes in the neuropathic bladder. However, as the authors have not studied expression of cytokeratin 20 in TCC cases of the neuropathic bladder, this conclusion really cannot be drawn. The more obvious conclusions are that there appears to be an absence of cytokeratin 20 in a high proportion of urothelium from neuropathic bladders. The reasons for this could be either that there is an underlying metaplasia or that changes in the neuropathic bladder affect urothelial differentiation. This question could be addressed by further analysis of other differentiation markers of urothelium, including cytokeratins and uroplakins. Taken with evidence from other systems, such as loss of cytokeratin 20 expression from static organ cultures (Scriven et al., 1997), this might suggest that other factors, such as mechanical stimulation of the urothelium, could affect expression of markers such as cytokeratin 20.

Acknowledgement
17. This is entirely inappropriate and should be deleted.

Tables
18. These are not referred to in the text. The authors should remove the biopsy and histology numbers and year of birth of patients and refer instead to an anonymised number and an age.
Competing interests:

None declared.