Reviewer's report

Title: Total and caspase-cleaved cytokeratine 18 in chronic cholecystitis: A prospective study

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Reviewer: Stig Linder

Reviewer's report:

Simopoulos et al., "Total and caspase-cleaved cytokeratin 18 in chronic cholecystitis: A prospective study".

This is a rather preliminary but potentially interesting study on detection of caspase-cleaved CK18 products in bile samples.

The levels of caspase-cleaved CK18 in the bile of both groups of cholecystitis patients is high (5 - 10-fold) compared to the levels observed in serum from the same patients (and from normal individuals as reported in the literature). This finding may indicate elevated apoptosis in the epithelial cells of the gall bladder. It is difficult to interpret the finding since the levels of caspase-cleaved CK18 in bile fluid from normal subjects is unknown. If it is not possible to obtain bile from normal individuals, the study is therefore very not conclusive.

How were measurements in bile performed? The M30/M65 ELISA assays have not previously been used for bile samples and assay performance, CK18 solubility etc (and, in addition, dilution and spike recovery characteristics) are not known. Were any assay validation studies performed (efficiency of detection of exogenously added assay standard to bile fluid, dilution linearity of bile samples)?

The M30 antibody was used for immunohistochemistry (Fig. 1). The experiment is refereed to as "intense expression of CK18 was presented in the epithelial cells of the mucosa of the gall bladder". This is misleading - the authors must mean to say "cells staining positive for caspase-cleaved CK18 were present...". However, I do not see the staining pattern expected (occasional apoptotic cells staining positive using the M30 antibody) in the figure (all cells can not be positive - apoptosis is a transient phenomenon and the epithelium would not be maintained if this was so). Positive cells should be indicated with arrows. Again, there is no comparison to gall bladder from subjects not suffering from cholecystitis (which I understand may be difficult to obtain).

This study would be interesting if it is possible to perform IHC on normal gall bladder epithelium, or obtain gall samples from subjects not suffering from cholecystitis. If this is not possible, the study appears to be of limited value.

Minor issues:
1. There are some spelling errors; caspace (caspase), cytokeratine (cytokeratin).
2. Page 3: "liberating neo-epitopes inaccessible to thr caspase cleavage site" (what does this mean?)

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I have a financial interest in the company that produces the ELISA kits used for this study (Peviva AB).