Reviewer's report

Title: Lymphatic marker podoplanin/D2-40 in human advanced cirrhotic liver- Reevaluations of microlymphatic abnormalities

Version: 1 Date: 14 September 2010

Reviewer: Patricia Lalor

Reviewer's report:

- Major Compulsory Revisions

i) The manuscript describes an interesting study to determine the validity of using an antibody raised against podoplanin to identify lymphatic vessels in human liver samples, and goes on to generate data describing how the density of lymphatics changes with disease. The authors assert that this is the first study to quantify the density of lymphatic vessels in cirrhosis and demonstrate expression of podoplanin in cirrhosis. Staining with Cd34 is used throughout to differentiate ‘vascular’ from ‘lymphatic’ endothelium.

I would suggest that given the broad readership of this particular journal the authors should expand the introduction to discuss the availability and specificity of different markers for the different components of the vascular tree in the normal and diseased liver. This is particularly important in the context of CD34 expression for example which is ‘mostly’ present on ‘vascular’ endothelium in the liver but has been reported on lymphatics in some studies (some of which include expression on tumors which arise on a cirrhotic background), does stain sinusoidal endothelium when it becomes ‘capillarised’ in disease (in support of some of the data shown in periportal images in this manuscript), and has also been reported on various precursor cell populations in the liver. The authors observations on the extent of CD34 expression are therefore not novel and they should temper their discussion relating to links between CD34 and podoplanin expression. For example in Fig 1c the CD34 staining at low power nicely highlights positivity on central vessels and portal vessels in normal tissue, but there is also a suggestion of some weak stain on a structure that may be sinusoidal at the centre of the lower half of the panel. Thus clearer discussion of the true identity of cells bearing increased CD34 expression in cirrhotic samples (eg in the western blot samples used in Fig2a) should be included.

ii) The authors should check their statement that they are the first to determine lymphatic vessel density in cirrhosis. This may indeed be the case using this particular marker but I believe there may be other studies characterising density using other means including vascular casts. This should be clarified.

iii) Similarly it would be useful to have a little more clinical data for the ‘normal’ samples used. It is suggested these are non-involved samples from tumour patients I believe, and thus there may be potential for modulation of vascular phenotype in fibrotic samples (however I acknowledge that the morphology on
the histological staining images shown looks good).

- Minor Essential Revisions
i) Given potential for sections used during image analysis to contain various vascular compartments across the different zones of the lobule, and variations in size of portal areas with advanced cirrhosis it would be helpful to add detail as to how the scoring was performed (ie how were areas chosen for scoring, are the same amount of vessels included in each ‘representative’ field, were only portal fields considered ?)

ii) Western blot figure suggests GAPDH blotting used for densitometric comparison of samples but methods section suggests b-actin was used. Should be clarified

iii) Electron micrographs are beautiful but quite difficult for a non-expert to interpret purely on morphological appearance, a few additional labels might be helpful.

iv) Labelling on figure panels is a bit confusing as each page bears a figure number(1-9) whilst the correct figure numbers are also stated in black text on the panels. Should be corrected for final version.

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

Quality of written English: Needs some language corrections before being published

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

Declaration of competing interests:

No competing interests