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Letter to the Editor

Immunohistochemical pitfalls in the diagnosis of hepatocellular adenomas and focal nodular hyperplasia: accurate understanding of diverse staining patterns is essential for diagnosis and risk assessment

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To the editor: We thank Bioulac-Sage *et al*[1] for their commentary in response to our publication.[2] This group has done seminal work regarding the immunohistochemical and molecular features of hepatocellular adenoma and focal nodular hyperplasia. We agree with their previously published data, but it appears that some of our immunohistochemical data has not been clearly represented in their commentary. As the proper interpretation of immunohistochemistry is crucial in the biopsy diagnosis of hepatocellular adenoma and focal nodular hyperplasia, the following comments are being made for clarification.

Bioulac-Sage *et al*[3] have stated that the immunohistochemical criteria used in our study have not been validated and recommend that the criteria validated by them using molecular data should be used. This observation is surprising as our criteria are based on those recommended in their study.[3] For example, diffuse staining with glutamine synthetase was defined in our study by staining of >50% of tumor cells and all these cases were thus interpreted as β-catenin-activated lesions. The 50% cutoff was chosen on the basis of the results of the study by Bioulac-Sage *et al*.[3] They had described homogeneous and heterogeneous patterns of glutamine synthetase staining within this group, but did not provide the number of cases for each category. In our study,[2] we also observed homogenous and heterogeneous patterns within the diffuse glutamine synthetase staining group and described them as diffuse-strong and diffuse-intermediate staining, respectively; in addition, we also provided number of cases showing these two patterns within the diffuse category.

Our study also describes two additional patterns of glutamine synthetase staining in hepatocellular adenoma: perivascular, and perivascular with patchy staining. Bioulac-Sage *et al* have stated in their commentary that these patterns are 'confusing and have no sustained meaning supported by molecular data'. The descriptions of the terms 'perivascular' and 'perivascular with patchy staining' are detailed in the methods section of our study. Hence, we are of the opinion that there should not be any cause for confusion. In fact, Bioulac-Sage *et al* have described the same patterns in their studies. Our description of 'perivascular and patchy staining' with glutamine synthetase corresponds at least in part to the description of 'patchy staining' with glutamine synthetase in their own studies.[3,4] Furthermore, our study is focused on a detailed description of the observed immunohistochemical staining patterns, which simulates the situation for majority of pathologists who encounter these lesions in practice. Molecular techniques were not part of our study and we have not claimed any molecular correlations based on our data. On the basis of current understanding, 'patchy' or 'perivascular and patchy' glutamine synthetase staining does not correlate with exon 3 *β-catenin* mutations, and this pattern should not be considered a high-risk feature while making management decisions. As Bioulac-Sage *et al* have stated in their commentary, further molecular studies are necessary to understand the significance of the wide range of glutamine synthetase staining patterns observed in hepatocellular adenoma, and we concur with their assessment that these recommendations may change in the future.

In our study, we also described a 'pseudomap-like staining' with glutamine synthetase that was observed at the periphery of some hepatocellular adenomas. This can be mistaken for map-like staining pattern typically seen in focal nodular hyperplasia, especially in needle biopsies. We provide a detailed histologic description of how to avoid this pitfall. In their commentary, Bioulac-Sage *et al* state that this pattern is a 'real problem' and that 'this should not lead to a specific category of lesions until proven to be related to beta-catenin mutations by molecular biology'. We want to emphasize that we have not stated in our paper that this pattern defines a specific category of lesions, but have highlighted this pattern to avoid the erroneous diagnosis of focal nodular hyperplasia when the periphery of a hepatocellular adenoma has been sampled on biopsy. In fact, a similar description of enhanced glutamine synthetase staining at the periphery of the hepatocellular adenoma has been noted in a previous study by Bioulac-Sage *et al*, although the pattern was not discussed in detail.[5]

For serum amyloid-associated protein staining, we considered positive cases as being diffuse

(>50%) or focal (10–50%). In their commentary, Bioulac-Sage *et al* state that 'the staining is positive (from mild to strong) or negative; diffuse positivity means close to 100% positivity and focal positivity means expression around inflammatory cells, which is not considered significant'. However, the results in their prior study[3] indicate that serum amyloid A staining was not diffuse in all cases and was described as being 'patchy and/or faint'; the number of cases with patchy and/or faint staining was not specified. We think that it is important to examine 'focal' *vs* 'diffuse' pattern of staining to assess how well a marker will work in the setting of a needle biopsy when only a small portion of the lesion is available for immunohistochemical evaluation. Whether the term 'focal' should be used to refer to staining of 10–50% of tumor cells is a minor semantic point, and should not cause any confusion if the description of the term is clearly stated in the methods section of the study.

Bioulac-Sage *et al* have stated that they consider C-reactive protein as being more reliable than serum amyloid A for the diagnosis of inflammatory hepatocellular adenoma. In their original paper, details of C-reactive protein staining are not provided, whereas serum amyloid A staining has been described in detail for the diagnosis of inflammatory hepatocellular adenoma.[3] Their subsequent publications also recommend serum amyloid A or C-reactive protein for the diagnosis of inflammatory hepatocellular adenoma, and do not provide evidence for the superiority of C-reactive protein.[6,7] In our experience, serum amyloid A has better specificity than C-reactive protein for the diagnosis of inflammatory hepatocellular adenoma.[2] C-reactive protein has higher sensitivity, but it is frequently positive in variable patterns in focal nodular hyperplasia and thus can be mistaken for positivity seen in inflammatory hepatocellular adenoma, especially in needle biopsies.

We agree with Bioulac-Sage *et al* that the interpretation of glutamine synthetase, serum amyloid A, and C-reactive protein staining can be challenging, and a discussion on the interpretations and pitfalls of different staining patterns among experts, as well as correlation with molecular data, is essential for the accurate diagnosis of hepatocellular adenoma and focal nodular hyperplasia.

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