Reviewer's report

Title: Complement activation in astrocytomas: deposition of C4d and patient outcome.

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Reviewer: Craig Horbinski

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The manuscript titled “Complement activation in astrocytomas: deposition of C4d and patient outcome” by Makela et al. studies the expression of C4d complement product in glioma cells and their vascular beds. They show a correlation between the strength of C4d and WHO grade, although PAs also have a fairly high level of C4d.

This subject seems to be novel, and the morphologic/immunohistochemical analyses are fairly rigorous, attempting to adjust for overall tumor vascularity. However, the overall N is rather modest, the clinical information on these cases is sparse, and the Discussion is full of overaggressive conclusions from these descriptive data.

Major compulsory revisions:

1. Were the recurrent gliomas previously exposed to adjuvant radiochemotherapy, which might alter their C4d expression? It seems that analysis of primary and recurrent tumors should also be split apart, to get a clearer picture of what primary C4d expression patterns are like.

2. While increased C4d might correlate with worse outcome, it is not clear that it could be an independent prognosticator since multivariate analysis was not done, taking into account WHO grade. Thus, the significance of Figure 5, and its related conclusions in the Discussion, are diminished.

3. Much of the Discussion is highly speculative, drawing causative conclusions from their correlative data. For example, “diffusely infiltrating astrocytomas form probably more inflammatory mediators in a particular period of time, since they have faster proliferation and cell death rates” should be deleted, since it is purely speculative and cannot be supported by the data. Many slow-growing brain tumors, including PAs, can also elicit brisk inflammatory responses. Likewise, “Thus the pathological microenvironment that inflammation provides is the most likely aspect to explain the survival study results, which showed that a worsened outcome is associated with intensified complement activation in diffusely infiltrating astrocytomas” is not proven by this correlative study, especially since no multivariate analyses were done to adjust for WHO grade. “C4d positivity in diffusely infiltrating astrocytomas could predict the recurrence after resection and also the malignant progression of these tumors” is also not justified by the data. All these things might be true, but such unequivocal conclusions as are presented in the Discussion need to be toned down.
4. Along these lines, have the authors considered the possibility that C4d accumulation is merely a secondary event to other better-characterized phenomena, such as robust microvascular proliferation causing poorly-formed, leaky blood vessels that might elicit some inflammatory changes? For example, are the authors aware that microvascular proliferation is a feature of both grade I PAs and grade IV GBMs, which is why both tumors routinely show contrast enhancement on MRI?

Minor essential revisions:

1. Cutoffs for C4d in the glioma cells seem to be rather low. Is this typical for C4d expression in other cancers and tissue types?

2. How can the authors be certain that “moderate diffuse cytoplasmic” staining (e.g. figure 1) isn’t just nonspecific background staining?

3. In Figure 4, use Roman numerals when referring to WHO grade (i.e. I-IV not 1-4).

4. The authors should cite Gousias et al. (J Neuroimmunol. 2010 Sep 14;226(1-2):136-42), as this group showed reduced serum C4 in patients with glioma and an inverse correlation between serum C4 and glioma grade. Perhaps this is because all the complement is being “consumed” by hypervascularized gliomas?

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

I declare that I have no competing interests.