Author's response to reviews

Title: Prolactin gene expression in primary central nervous system tumors

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Author's response to reviews: see over
Dear Dr. Susanne Brummelte,

In response to the comments of the reviewers, the following changes were performed (in red in the text):

**Reviewer number: 1**

Major Compulsory Revisions: none  
Minor Essential Revisions: none  
Discretionary Revisions: Authors suggest that if PCR for PRL is negative than this is the gold standard and PCR is 100% true. However for clinicians this could be discussed is the discussion section after the discussion on the immunhistochemistry part, authors could include some papers that support the fact that PCR is 100% and a negative PCR may not be due to any technical artefacts or so.

1. We have increased the Discussion, fourth paragraph: “Real time -PCR is the most sensitive method for the detection and quantification of mRNA, especially for low abundance specimens, like RNAs, cells and tissues [27, 34]. Real time-PCR assays are 10.000 to 100.000-fold more sensitive than RNase protection assays, 1000-fold more sensitive than dot blot hybridization, detecting a single copy of a specific transcript and is a method with lower variation, usually between 0 and 5% for TaqMan probes [27-29, 34, 35]. The real time -PCR technique, specially with TaqMan probes, is the best option when analyzing gene with low expression level, from limited samples once high sensitivity and accuracy are needed [27, 36].”

We have provided published papers that support the fact that PCR is the most sensitive method for the detection and quantification of mRNA, detecting a single copy of a specific transcript. See Discussion, fourth paragraph. See References (34-36).

34. Mackay IM. **Real-time PCR in the microbiology laboratory.** *Clin Microbiol Infec* 2004, 10:190-212.
2. Supporting that negative PCR was not due to any technical artefacts, we have added in Materials and Methods section the following data:

**PCR-RT:**
- First paragraph, last sentence:
  “The same procedures were adopted for the pool of normal peritumoral tissue as well as the prolactinoma used as positive control.”
- Third paragraph, first sentence:
  “A pool consisting of 9 samples of normal peritumoral tissue (gray and white matter CNS and meningothelial tissue) was used as calibrator to allow comparison between CNS tumoral and normal samples.”
- Third paragraph, third sentence:
  “The tests with CNS tumor samples were performed in duplicate, and the GAPDH gene and PRL gene were always tested in the same assay, avoiding possible differences between assays.”
- Third paragraph, forth sentence:
  “The GAPDH gene was detected in all samples.”
- Third paragraph, fifth sentence:
  “The PRL gene was detected in the prolactinoma sample (positive control) in all assays.”
- Third paragraph, last sentence:
  “In each reaction plate and for each gene tested, the pool of normal CNS tissue samples (calibrator) was performed in triplicate, the positive control (prolactinoma) and negative control in duplicate.”

3. We have reviewed all the text and modified the references and their numbers after the item 15. See Text and References (15-47).

4. Observation: reference 14 was published as a complete paper, so we have modified the reference: DMD Abech, JFS Pereira-Lima, CGS Leães, RT Meurer, Barbosa-Coutinho LM, NP Ferreira, MC Oliveira. **Cell replication and angiogenesis in central nervous system tumors and their relationship with the expression of tissue prolactin and hyperprolactinemia.** *Open Journal of Pathology* 2012,2:50-57.
Reviewer number: 2
Reviewer's report: There are no corrections to be made.

We look forward to hearing from you regarding the status of our manuscript. In the meantime, please feel free to contact us if you need any additional information.

Sincerely yours,

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