Author Response: Comments on Assays Used to Measure VEGF in the Presence of Anti-VEGF Therapeutics

We wish to thank the authors for their letter[1] and comments on our paper.[2] In our paper, we clearly pointed out VEGF concentration is underestimated by ELISA assay in the presence of anti-VEGF drugs and suggested that investigators should exercise caution when interpreting measurements of VEGF ELISA in patients being treated with an anti-VEGF drug. The authors concur with us. In a recent paper the authors cited, one of the authors states, "as noted by the manufacturers of this kit, this assay (i.e., ELISA assay kit they used for their study) is not suited to measure total VEGF in the presence of anti-VEGFs, because of the binding properties of the capture and detection reagents used in the assay. However, this assay is commonly used for measuring free-VEGF levels in samples from patients treated with anti-VEGF therapeutics."[3]

The authors point out the importance of measuring "free" or unbound VEGF concentrations. In our *IOVS* paper, we also implied that ELISA does not measure the free-VEGF concentrations in the presence of anti-VEGF antibody either. The authors mention that literature-derived Kd values we used may not be appropriate to derive free-VEGF concentrations. However, neither their paper[4] nor our paper support the usefulness of ELISA assays in assessing the level of unbound VEGF in the presence of anti-VEGF drugs (to assist in the assessment of their therapeutic and safety impacts).

The paper by Yang et al.[4] used a Biacore system to evaluate the relative binding and potencies of three inhibitors of VEGF, used to treat neovascular AMD, and assessed their relevance in the context of clinical outcome. They concluded that aflibercept did not bind with higher affinity than ranibizumab to VEGF. We agree that we need to be careful in interpreting data on biomolecular interactions. The variability of Kd may be due to various factors, such as the assay format used. We actually noted that other studies do not support their results.[5,6] The authors also mention that VEGF/anti-VEGF complexes in the samples diluted into an assay will tend to dissociate and have more free-VEGF, and therefore, assume that the ELISA assays will tend to overestimate free-VEGF levels. However, this assumption is incorrect, because anti-VEGF drugs in the samples may interfere with VEGF ELISA measurements (see Fig. 1 in our original article[2]) and ELISA assays do not measure the concentration of "free" or unbound VEGF. This is clearly mentioned in our original article. There is also a possibility that ELISA detects VEGF/anti-VEGF complex.

Therefore, for now, we believe it would be prudent to consider measured concentration is not the same as the free-VEGF concentrations. Further studies are needed to address whether ELISA assays are really useful in assessing "free"-VEGF levels in the presence of anti-VEGF drugs. Until then, clinicians should exercise caution when interpreting measurements of VEGF ELISA in patients being treated with an anti-VEGF drug.

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