Platelet Normalized Serum Vascular Endothelial Growth Factor Levels in Progressive Pediatric Solid Malignancies

We read with interest the recently published article by Pramanik, *et al.* [1]. The study reported inconsistent trends in serum vascular endothelial growth factor (VEGF) and thrombospondin-1 (TSP-1) in 108 patients with progressive pediatric solid tumors who received metronomic chemotherapy or placebo [1]. While higher baseline serum VEGF levels predicted inferior overall survival, authors found that responders with metronomic chemotherapy had significantly lower VEGF levels at baseline compared with non-responders. Further, there was no association of serial VEGF levels with response to metronomic chemotherapy.

Of note, VEGF is released from the α granules on platelet activation during sample collection and therefore, serum levels are considered as an inaccurate indicator of actual measurement of circulating VEGF [2]. Patients with disseminated cancer may have a higher platelet count and carry even higher VEGF per platelets compared with general population [3]. Thus, plasma is preferred over serum to measure VEGF because collecting blood in citrate tubes avoids platelet activation and therefore preventing the spurious high VEGF levels released from platelets [4]. Since the authors used serum to measure VEGF levels in pediatric patients with solid tumors in the study, the results must be interpreted with caution [1]. However, serum VEGF levels normalized to patient's platelet count provides serum VEGF/platelet, which can neutralize the effect of VEGF released from platelets while withdrawing blood [5]. Therefore, the authors may consider analyzing the data after calculating serum VEGF/platelet for all measurements in individual patients, if data on platelet count is available. It will be interesting to see if a consistent pattern is then noticed between serum VEGF/platelet with the response to metronomic chemotherapy and survival outcomes.

Further, the authors described the effect of baseline serum VEGF levels with overall survival in overall population as well as responders in patients randomized to metronomic chemotherapy arm [1]. While this finding is interesting, it is an exploratory subgroup finding in a small number of patients, which can be interpreted as hypothesis generating at best and therefore, should be interpreted with utmost caution.

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AUTHORS' REPLY

We appreciate the comments and suggestions by the reader. Available literature shows that there is a controversy regarding the best blood compartment and the best test to measure VEGF in cancer patients. VEGF in cancer patients is the sum total of platelet derived VEGF as well as other sources like neo-angiogenesis in the tumor tissue. One of the studies showed that the best discrimination between healthy volunteers and cancer patients was observed in platelet poor plasma (PPP). As generating plasma induces platelet activation with consequent VEGF release from platelets, citrate-theophylline-adenosine-dipyridamole plasma was suggested by some authors to evaluate VEGF [1]. Serum VEGF is more practical because VEGF levels in citrated plasma are low and lie close to the limits of ELISA sensitivity. Some studies have shown that a standardized measurement of serum VEGF, normalized by the patient's platelet count, which gives a value of serum VEGF per platelet, can be a useful parameter [2].

We had our baseline platelet counts for all the patients but the corresponding platelet counts for subsequent follow up (A2 and A3) assessments were not available for all patients [3]. Hence, we restricted our analysis to baseline values only. On applying pair wise correlation to the baseline platelet count and serum VEGF, we found an insignificant correlation; r=0.16 (P=0.09) (***Fig.* 1a**). Baseline serum VEGF showed a significant positive correlation with baseline VEGF per platelet (r=0.81, P<0.0001) (***Fig.* 1b**). As the serum VEGF and VEGF/per platelet correlate significantly, both are likely to follow similar trends; this implies that we are likely to have similar observations, whether we use serum VEGF or VEGF/platelet.

Similar observations were reported by Vermeulen, *et al*. [4]; they commented that in view of the lack of a strong association