

## Commentary

## Modern prognostic factors and angiogenesis in chronic lymphocytic leukemia: More data needed

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**Abstract**

Angiogenesis appears to be an important player in biology of chronic lymphocytic leukemia (CLL). We present here data on association of ZAP-70 expression measured by flow cytometry and plasma levels of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) in 27 untreated CLL patients. We found significantly higher VEGF (but not bFGF) in ZAP-negative patients. Likewise, there was a negative correlation between percentage of ZAP-70 expression and VEGF. Larger, prospective studies are needed to confirm our pilot data.

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Molica et al. [1] recently published their data on angiogenic markers and their relationship to prognostic factors in chronic lymphocytic leukemia (CLL). Because the authors state that “reports addressing the relationship between angiogenic phenotype of CLL and new biological parameters of prognostic relevance such as IgV<sub>H</sub> gene mutational status and expression of ZAP-70 are absent”, we would like to draw their and readers’ attention to study by Shanafelt published in abstract form [2] and to our results [3]. While we found significantly higher *plasma* levels of bFGF (but not VEGF) in IgV<sub>H</sub>-mutated patients, Shanafelt’s study failed to show association of IgV<sub>H</sub> mutational status or CD38 expression with *serum*, cellular or secreted levels of VEGF, bFGF or thrombospondin-1 in a large patient cohort. Results reported by Molica et al. show that serum VEGF (but not bFGF or microvessel density) correlated with IgV<sub>H</sub> muta-

tional status, CD38 and ZAP-70 expression in 23 CLL patients. In our own series we compared ZAP-70 expression with plasma levels of bFGF and VEGF in 27 untreated CLL patients (18 males, 9 females, median age 64 years [range, 31–83], Rai stage 0/I/II in 14/10/3 patients). ZAP-70 was quantified using phycoerythrin-conjugated antibody (clone 1E7.2, Caltag Laboratories, Burlingame, CA), isotype control for positivity threshold and 20% positivity cut-off. Plasma bFGF and VEGF were measured using commercial ELISA Quantikine kits (R&D Systems, MN, USA). Ten patients were ZAP-70 positive and 17 ZAP-negative. Interestingly, we found significantly higher VEGF in ZAP-negative versus positive patients ( $p = 0.018$ , Mann-Whitney test); ZAP-negative patients had also higher bFGF levels but the difference did not reach statistical significance ( $p = 0.088$ , Mann-Whitney test). Similarly, we found a significantly *negative* correlation between plasma VEGF and ZAP-70 expression as a continuous variable ( $r = -0.53$ ,  $p = 0.0049$ , Spearman correlation) while relationship between bFGF and

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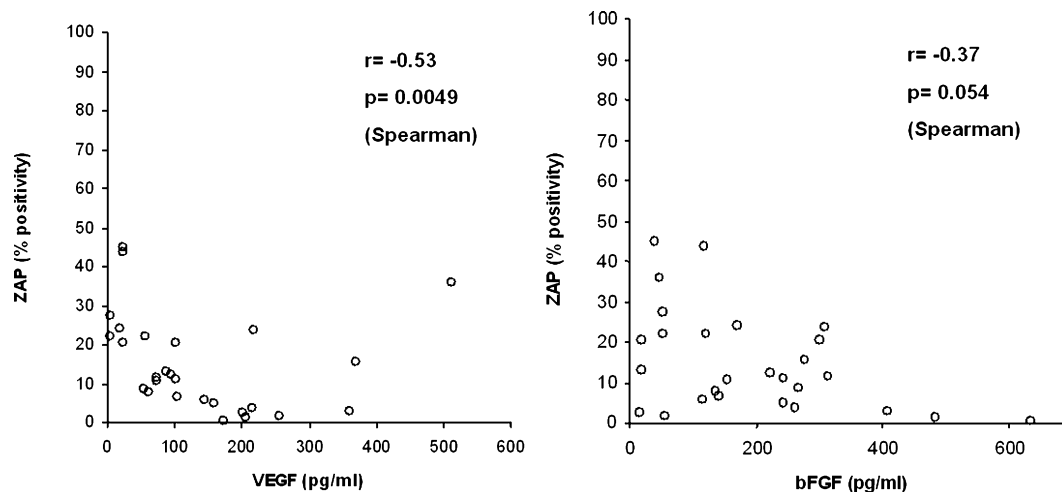


Fig. 1. Correlation between angiogenic cytokines and ZAP-70 expression in CLL patients ( $n = 27$ ).

ZAP-70 was of borderline significance ( $r = -0.37$ ,  $p = 0.054$ , Spearman correlation Fig. 1). There are two main reasons for these conflicting results. First (and perhaps most importantly), both studies dealt with limited number of patients, and selection bias is likely to be significant; therefore, both studies strongly need confirmation in a larger series. Secondly, the results are not directly comparable due to different material used (serum versus EDTA plasma) and different method for ZAP-70 quantification (FITC-conjugated antibody clone 2F3.2 versus PE-conjugated 1E7.2 antibody) which is a well-known problem precluding standardization. Taken together, these results point out the need for large prospective studies using comparable methods in order to further elucidate the significance of angiogenesis in CLL.

### Conflicts of interest

The authors state no relevant conflict of interest.

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### References

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