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## Elevated serum vascular endothelial growth factor levels in patients with polycythemia vera and thrombotic complications

Vascular endothelial growth factor (VEGF) induces platelet activation in a thrombin-dependent manner. We tested the serum VEGF levels in patients with polycythemia vera (PV) and found a significant correlation between increased VEGF and thrombosis. These findings suggest that high VEGF levels might contribute to the occurrence of thrombosis in this hematologic malignancy.

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The thrombotic risk remains difficult to predict in patients with polycythemia vera (PV). Bellucci *et al.*[1] studied β-TG and PF4 levels in patients with normal platelet counts receiving antiaggregant and cytoreductive therapy. These authors failed to establish a clear correlation between increased β-TG and PF4 and thrombosis.[1] On this basis, we evaluated platelet function and number in a series of patients with polycythemia and tried to correlate the results with a history of thrombosis. Increased bone marrow angiogenesis has been demonstrated in patients with PV.[2] Recent studies indicate that vascular endothelial growth factor (VEGF), the major stimulus of angiogenesis, promotes platelet adhesion and activation in a thrombin-dependent manner and that on activation the platelets release VEGF.[3] Thus, we also measured VEGF levels.

The study group comprised 19 patients (13 men, 6 women; mean age 63.1 years [range 20-81]) suffering from PV, as defined by the Polycythemia Vera Study Group criteria.[4] Their mean duration of disease was 5 years (range 2-12). A group of 10 healthy subjects (3 men, 7 women; mean age 55.3 years [range 35-85]) acted as controls. Sixteen out of nineteen patients received hydroxyurea and antiaggregant agents, either aspirin or ticlopidine, indobufen and dipirydamole. The remaining three were managed with phlebotomy alone. Thrombotic complications had occurred in 8 patients (5 men, 3 women; mean age: 65.38 years) and included two episodes of deep vein thrombosis, two of transient ischemic attacks, two of myocardial infarction and two of microvascular thrombosis of extremities (erythromelalgia) (symptomatic group). The other eleven patients (7 men, 4 women; mean age: 60.55 years) had not experienced thrombosis (asymptomatic group). We excluded the presence of both acquired thrombotic risk factors (hypertension, smoking, obesity, hyperlipidemia, antiphospholipid syndrome) and inherited ones (antithrombin III, protein C and protein S deficiency, factor V Leiden, prothrombin G20210A and MTHFR C677T mutations). Serum VEGF and plasma β-TG and PF4 were measured by ELISA (Quantikine Human VEGF Immunoassay, R&D Systems, Minneapolis, MN, USA; Diagnostica Stago Boehringer Mannheim, Germany). To be sure that platelets did not release VEGF differently during the preparatory steps, depending on whether they came from a patient with a history of thrombosis or not, in 19 patients VEGF measurement was subsequently repeated in another sample collected after 1 or 2 months. These measurements showed concordance for VEGF concentration. The time elapsed between venipuncture and centrifugation for VEGF was 30 min. Platelet counts were determined by a Sysmex SF-3000 analyzer (Dasit, Milan, Italy). The results are summarized in Tables 1 and 2. All patients had significantly higher values of β-TG and PF4 than did the controls ($p<0.001$) and normal platelet counts. Of these 19 patients, 8 exhibited significantly higher VEGF levels than the normal subjects ($p<0.0001$) and had experienced thrombosis, whereas 11 showed normal VEGF levels and had not had thrombosis.

A significant difference was observed in VEGF levels between symptomatic and asymptomatic patients ($p<0.0001$, Student's t-test). A highly significant correlation was found between VEGF levels and thrombotic events estimated as Spearman's coefficient ($p<.0001$). No correlation was found between increased VEGF and platelets. In agreement with Bellucci *et al.*,[1] we found no correlation between elevated β-TG and PF4 and thrombosis.

**Table 1. Bioclinical data of 19 patients with PV.**

| *Patient No.* | *Age/Sex* | *βTG 10-40 IU/mL** | *PF4 0-5 IU /mL** | *Platelets 150-450 ×10⁹/L** | *VEGF 62-707 pg/mL** | *Thrombotic events* | *Concomitant therapy* |
|---|---|---|---|---|---|---|---|
| 1 | 78/F | 180 | 85 | 305 | 621 | No | Hu+IND+DYP |
| 2 | 47/M | 220 | 90 | 262 | 228 | No | Phlebotomy |
| 3 | 37/M | 350 | 245 | 227 | 556 | No | Phlebotomy |
| 4 | 69/M | 210 | 140 | 547 | 1119 | Yes/TIA | Hu+IND+DYP |
| 5 | 65/F | 240 | 235 | 310 | 1354 | Yes/DVT | Hu+ASA |
| 6 | 53/M | 260 | 245 | 522 | 1506 | Yes/E | Hu+ASA |
| 7 | 60/M | 150 | 90 | 301 | 225 | No | Hu+ASA |
| 8 | 70/M | 246 | 97 | 245 | 1366 | Yes/MI | Hu+Ind |
| 9 | 71/F | 243 | 106 | 292 | 392 | No | Hu+ASA |
| 10 | 67/F | 200 | 95 | 320 | 1845 | Yes/DVT | Hu+ASA |
| 11 | 79/M | 250 | 104 | 230 | 444 | No | Hu+ASA |
| 12 | 81/M | 230 | 120 | 299 | 791 | Yes/E | Hu+ASA |
| 13 | 71/F | 240 | 95 | 564 | 1510 | Yes/TIA | Hu+TIC |
| 14 | 20/M | 220 | 198 | 223 | 575 | No | Phlebotomy |
| 15 | 69/M | 245 | 480 | 357 | 671 | No | Hu+IND+DYP |
| 16 | 57/M | 210 | 180 | 230 | 848 | Yes/MI | Hu+ASA |
| 17 | 74/M | 200 | 200 | 217 | 357 | No | Hu+ASA |
| 18 | 59/M | 240 | 118 | 374 | 638 | No | Hu+ASA |
| 19 | 81/F | 246 | 106 | 423 | 591 | No | Hu+ASA |
| Mean° | 63.11 ±15.69 | 230.531 ±39.86 | 59.42 ±96.17 | 328.84± 110.82 | 823± 482 | | |
| Controls° | 55.3± 18.81 | 26.0± 8.59 | 2.68± 1.14 | 244.4± 66.86 | 194± 180 | | |

**In brackets normal range; °Values shown are means±SD. TIA: transient ischemic attack; DVT: deep vein thrombosis; E: erythromelalgia; MI: myocardial infarction.*

**Table 2. Statistics of 19 patients with PV.**

| | *Patients with thrombosis (n=8)* | *Patients without thrombosis (n=11)* | *p* |
|---|---|---|---|
| VEGF | 1292±355 | 481±161 | <.0001 |
| VEGF/Thrombosis | | | <.0001 |
| VEGF/Platelets | | | NS |

Interestingly, we did reveal an association between increased VEGF levels and thrombotic complications.

An increase in either intratumoral angiogenesis or serum levels of VEGF has been demonstrated in a variety of hematologic malignancies.[5] The possible prognostic relevance of these measurements in chronic myeloproliferative disorders (CMD) has been reported by Musolino *et al.* who found significantly higher VEGF levels in CMD patients with vascular complications.[6] It has also been reported that tumor-released VEGF activates endothelial cells to become prothrombotic causing platelet adhesion and activation.[3] Musolino *et al.* observed endothelium activation in CMD patients and thromboembolic complications.[7]

Therefore, it is speculated that increased VEGF might be responsible for the endothelial activation. Other authors have reported increased VEGF in CMD accompanied by thrombocytosis that could limit the interpretation of this finding since platelets are a major source of VEGF.[8] We found increased VEGF levels in patients with PV without thrombocytosis and this increase was not correlated with the platelet count.

Thus, in our study increased levels of VEGF might reflect a state of platelet activation and be responsible for occurrence of a thrombotic event. We, therefore, think that VEGF measurement might have a clinical use in categorizing high- and low-risk PV patients and be appreciated as an additional variable in clinical prognostic models. These data, if confirmed in larger studies, might also support the rationale for using angiogenesis inhibitors as antithrombotic agents.

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## t(15;17) in acute promyelocytic leukemia is not associated with submicroscopic deletions on der(17)

We report a fluorescent *in situ* hybridization (FISH) study on 34 patients with acute promyelocytic leukemia. The study was designed to detect microdeletions in the derivative chromosome 17 which is the result of a reciprocal translocation t(15;17). No deletion was found.

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Acute promyelocytic leukemia (APL) is characterized by the reciprocal translocation t(15;17)(q22;q21), disrupting the PML and RAR-$\alpha$ genes, which are localized on chromosomes 15q22 and 17q21, respectively. The t(15;17) generates two chimeric genes: PML/RAR$\alpha$ arises on der(15), whereas the reciprocal RAR$\alpha$/PML fusion is located on the der(17).[1-2] Microdeletions on the derivative chromosome carrying the reciprocal fusion gene have been recently reported in some leukemia translocations such as t(9;22) in chronic myeloid leukemia (CML) and inv(16), as well as in t(8;21) and 11q23 abnormalities in acute myeloid leukemia (AML).[3-7] In CML Ph$^+$ and AML with inv(16) these microdeletions were associated with a worse prognosis. Microdeletions in APL cases have been recently investigated, by FISH, in 30 APL patients by Kolomietz *et al.*,[8] who utilized the Vysis LSI PML/RAR$\alpha$ translocation probe. This study did not reveal microdeletions. The Vysis probe, however, is not able to detect deletions in der(17), as it was specifically designed to detect the expressed fusion gene on der(15) chromosome. Therefore, their conclusion on the absence of deletions on der(17) has no experimental support. In contrast, we used appropriate FISH probes, specifically designed to detect deletions in der(17). Thirty-four APL patients were enrolled at diagnosis. All cases were tested by conventional cytogenetic and RT-PCR analysis to assess the presence of the t(15;17) and of the fusion gene PML/RAR$\alpha$, respectively. Co-hybridization FISH experiments were performed by using a mixture of two probes (BAC RP11-247C2 and PAC RP5-1112G21, from de Jong libraries), spanning the PML gene on chromosome 15 and RAR$\alpha$ gene on chromosome 17, respectively (Figure 1a). Their precise positions were derived from the Ensembl database (*http://www.ensembl.org/*). On metaphases of APL patients these probes generated two clear fusion signals on der(15) and on der(17) chromosomes in addition to single signals on normal 15 and 17 chromosomes (Figure 1b). The absence of a fusion signal on der(17) would reveal microdeletions on this chromosome. Twenty metaphases were evaluated for each patient. The analysis did not reveal any microdeletion, since an evident fusion signal on der(17) was observed in all analyzed metaphases.

In our series there was 1(3%) case with cryptic PML/RAR$\alpha$ fusion gene created by an insertional event, which is in agreement with the frequency of this abnormality in APL cases reported in literature.[9] In fact, this patient had apparently normal chromosomes 15 and 17 by conventional cytogenetic analysis while RT-PCR revealed expression of PML/RAR$\alpha$. Fluorescent *in situ* hybridization (FISH) experiments showed an abnormal hybridiza-