bolism in IAHD patients with APA can be excluded. Further studies with a longer follow-up and an increased number of patients and controls are therefore needed to exclude or confirm the risk of thromboembolism in these patients.

Velia Bongarzoni, Luciana Annino,* Andrea Roveda,° Maria Antonietta Amendolea,# Maria Cristina Tirindelli,@ Giuseppe Avvisati@*

**U.O. D. Ematologia, Ospedale "S. Giovanni-Addolorata"; °Dipartimento di Biotecnologie Cellulari ed Ematologia, Università "La Sapienza"; #Dipartimento di Malattie Infettive e Tropicali, Università "La Sapienza"; @Ematologia, Università Campus Biomedico, Roma*

Key words: idiopathic autoimmune hemolytic disease, thromboembolism.

Correspondence: Giuseppe Avvisati, MD, PhD, Ematologia, Università Campus Biomedico, via Emilio Longoni, 83 00155 Rome, Italy. E-mail: g.avvisati@unicampus.it

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Thrombosis

The role of D-dimer and residual venous obstruction in recurrence of venous thromboembolism after anticoagulation withdrawal in cancer patients

We assessed the predictive value of D-dimer (D-d) and residual venous obstruction (RVO), alone or in combination, for recurrent venous thromboembolism (VTE) over a 2-year follow-up in a cohort of 88 cancer patients after oral anticoagulant therapy (OAT) withdrawal following a first episode of proximal deep vein thrombosis of the lower limbs. RVO, determined by compression ultrasonography on the day of OAT suspension (T1), and abnormal D-d (cut-off value: 500 ng/mL), measured at T1 and 30±10 days afterwards, are independent risk factors for recurrent VTE in cancer patients.

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Cancer patients are considered at higher risk of recurrence after a first episode of venous thromboembolism (VTE) than are patients without cancer. As a result oral anticoagulant therapy (OAT) is recommended as long as the disease is active or in the case of chemotherapy.[1] However, cancer patients are at higher risk of bleeding during OAT[2,3] and markers of individual risk of recurrence could help tailor OAT duration. Recent studies have shown that residual venous obstruction (RVO) and D-

Table 1. Characteristics of patients and outcome events.

Evaluated: n.	*132*
Included : n. and sex	*88 ; m/f 35/53*
Excluded: n. and reasons for exclusion	*44 (32 for disease with metastases or requiring chemotherapy and/or radiotherapy, 6 for isolated PE and 6 for isolated distal DVT)*
Age (years)	Median (range)
All	71 (29-88)
M/F	70/71 (34-88/29-88)
Type and site of VTE	No. of cases
Proximal DVT	88
DVT + PE	12
right, left, bilateral DVT	37, 49, 2
Site of cancer	No. of patients
Breast	28
Prostate	12
Gastro-intestinal	13
Urogenital	9
Cerebral	2
Lung	2
Other	7
Hematologic	15
Duration of previous OAT	Months, median (range); mean
	6 (3-60); 8.8
Duration of follow-up: total (median)	122 years (17 months)
No. of months of previous OAT	No. of patients (%)
≤5 months, recurrences	30 (34.1), 9 (30%; 95% CI: 15-49%)
> 5 months, recurrences	58 (65.9), 12 (20.7%; 95% CI:11-33%)
Recurrences	23.9% (21/88) - 95% CI:15 -54%; 17.2% pt-years - 95% CI:11 -25% 4 isolated non-fatal PE 5 ipsilateral proximal DVT 12 contralateral DVT (1 distal), 2 with PE
Deaths	6 (6.8%) 4.9% pt -years; 95% CI:2 -10%
Abnormal D-d at T1*	19 (24.0%)
Recurrences	8 (42.1% ; 95% CI:20-66%)
Recurrences in the first 3 months	1
Abnormal D-d at T2*	34 (43%)
Recurrences	12 (35.3%;95% CI:20-54%)
Recurrences in the first 3 months	1
RVO present at T1	51 (57.9%)
Recurrences	16 (31.4% - 95% CI:19 -46%)
Recurrences in the first 3 months	6
Thrombophilic defects**: present	15 (22.3%)
FV Leiden	9
G20210A prothrombin mutation	4
Protein C deficiency	1
Protein S deficiency	1
Recurrences	5 (33.3%; 95% CI:12-62%)
Thrombophilic defects : absent	51
Recurrences	12 (23.6%; 95% CI: 13-38%)

** D-d levels were not available for 9 patients, of whom 2 had a recurrence.*
***Thrombophilia screening was not done in 22 patients (25%).*

Table 2. Recurrence rate for VTE according to the combination of D-d and RVO at T1 and T2 after OAT withdrawal.

	Normal D-d-without RVO	*Normal D-d-with RVO*	*Abnormal D-d without RVO*	*Abnormal D-d with RVO*
D-d and RVO at T1				
Recurrence rate% (95% CI) n./total	12 (3-31)° 3/25	23 (10-40) 8/35	14.3 (0-58) 1/7	58.3 (28-85) 7/12
% patient-years (95% CI) n./years	7.3 (2-20)@ 3/41	17.4 (8-31) 8/46	10 (0-44) 1/10	41.1 (18-67) 7/17
D-d at T2 and RVO at T1*				
Recurrence rate% (95% CI) n./total	0.0 (0-19)# 0/17	23.1 (9-44) 6/26	26.6 (8-55) 4/15	42.8 (22-66)^ 9/21
% patient-years (95% CI) n./years	0.0 (0-12)## 0/28	15.8 (6-31) 6/38	17.4 (5-39) 4/23	36 (18-57)^ 9/25

**No recurrences were observed between T1 and T2; °p=0.005 vs abnormal D-d with RVO; @p= 0.0044 vs. abnormal D-d with RVO; # and ## p=0.0002 vs. abnormal D-d with RVO; ^Hazard ratio for recurrence= 12.25 (95% CI: 1.5-100.3; p=0.02), after adjustment for age and sex, vs. normal D-d without RVO.*

dimer (D-d) levels are risk factors for recurrent VTE after OAT withdrawal;[4-8] however, cancer patients were usually excluded from these investigations. We set out to assess the risk for recurrent VTE conferred by D-d and RVO, alone or in combination, in a cohort of consecutive cancer patients after withdrawal of OAT prescribed for a first episode of symptomatic DVT of the lower limbs. Patients were enrolled on the day of OAT discontinuation and followed-up for two years. The institutional review board of S.Orsola-Malpighi University Hospital, Bologna, Italy approved the study. All subjects provided informed consent.

Inclusion criteria were a solid or hematologic (e.g. lymphoma, leukemia or multiple myeloma) cancer, an objectively documented first episode of proximal DVT, with or without pulmonary embolism (PE), OAT duration of at least three months, and ability to return for follow-up. Exclusion criteria were isolated iliac DVT or disease requiring prolonged OAT (e.g. cancer with metastases or requiring chemotherapy and/or radiotherapy, atrial fibrillation or known antiphospholipid syndrome). After at least three months of OAT, an oncology consultation was requested to establish disease inactivity on the basis of a standard series of radiological tests in case of a solid tumor and of bone marrow examination in case of hematologic malignancies. If disease was considered inactive, OAT was withdrawn and on the same day (T1), RVO was determined according to the method of Prandoni *et al*.[5] by compression ultrasonography (CUS). Venous blood samples were collected at T1 and 30+10 days (T2) afterwards from the antecubital vein into 0.129 mmol/L trisodium citrate. Plasma was prepared by centrifugation for 20 min at 2000 g at 20°C; plasma aliquots were snap frozen and stored at –70°C. D-d (cut-off value: 500 ng/mL) was measured by the VIDAS D-dimer ELISA method (BioMerieux, Lyon, France). Thrombophilia screening was performed at T2 as described elsewhere.[7] Periodic examinations were scheduled at 3, 9, 18 and 24 months after T1.

Patients were instructed to report immediately to the vascular emergency room in our department if they had symptoms of recurrent DVT and/or PE. Suspected DVT was evaluated according to Prandoni *et al*.[9] Suspected PE was diagnosed on the basis of objective algorithms.[10] DVT and/or fatal or non-fatal PE recurrences were considered as outcomes and adjudicated by two investigators (G.P. and B.C.) unaware of D-d, CUS and thrombophilia results.

Table 1 shows the patients' characteristics and outcomes. No patient was lost from the follow-up. The hazard ratio (HR) for recurrence associated with abnormal D-d at T1 and/or T2 was significant (4.01; 95% CI: 1.24-12.53; p=0.017), after adjustment for age, sex and RVO, when compared to persistently normal D-d at T1 and T2. The HR for recurrence associated with RVO was significant (3.8; 95% CI: 1.11-13.38; p=0.033), after adjustment for age, sex and D-d, when compared to absent RVO. RVO at T1 was combined with D-d both at T1 and T2 (Table 2) and a significantly higher recurrence rate was observed with abnormal D-d with RVO than with normal D-d without RVO. Our study has some limitations. We evaluated a small cohort, due to exclusion of a relevant number of patients, and D-d levels were unavailable for 9 subjects of whom 2 had a recurrence. However D-d and RVO did had significant effects, even after adjusting for confounding variables such as age or sex, albeit with large confidence intervals. Cancer-related factors such as type could not be evaluated. OAT duration could influence both D-d and RVO. However, OAT duration was similar among subjects with abnormal D-d at T1 and/or T2 and subjects with persistently normal D-d as well as among subjects with and without RVO.

We conclude that D-d and RVO are independent risk factors for VTE recurrence in cancer patients and may help tailor OAT duration. Abnormal D-d levels during and after OAT may reflect a state of hypercoagulability not suppressed by OAT, while RVO may also be a marker of a prothrombotic condition. A practical approach could be to withdraw OAT in case of normal D-d and absent RVO, measure D-d 1 month later and resume OAT in case of abnormal D-d. Such strategies should be addressed by appropriately designed management studies.

Benilde Cosmi, Cristina Legnani, Michela Cini, Giuliana Guazzaloca, Gualtiero Palareti

Department of Angiology and Blood Coagulation "M. Golinelli", S. Orsola-Malpighi University Hospital, Bologna, Italy

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Correspondence: Benilde Cosmi, MD, PhD, Department of Angiology and Blood Coagulation 'Marino Golinelli' S. Orsola-Malpighi University Hospital, via Albertoni 15, 40138 Bologna, Italy. Phone: international +39.051.6362301/2482. Fax: international +39.051.341642. E-mail: bcosmi@med.unibo.it/bcosmi@libero.it

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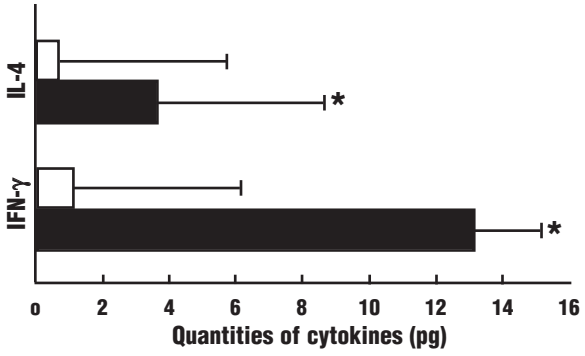

Figure 1. The quantities of type 1 (IFN-γ) and type 2 (IL-4) cytokines secreted by lymphocytes per microliter between G-PB and G-BM, the formula for the calculation of the cytokines as follows: quantities of cytokines=secretion of cytokines (pg/10^6 MNCs)×cell counts (MNCs/μL). *$p<0.001$, *independent t-tests was used (G-PB vs G-BM).*

Stem Cell Transplantation

A direct comparison of immunological characteristics of granulocyte colony-stimulating factor (G-CSF)-primed bone marrow grafts and G-CSF-mobilized peripheral blood grafts

Our preliminary results suggest the existence of quantitative and qualitative differences in immune cells and type1 and type2 cytokines between granulocyte colony-stimulating factor (G-CSF) primed bone marrow (G-BM) and G-CSF-mobilized peripheral blood grafts (G-PB). Our findings suggest that lower T-cell hyporesponsiveness and easier polarization of T cells from Th1 to Th2 are found in G-BM.

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Preliminary clinical trials have indicated that G-BM results in comparable engraftment, reduced severity of acute graft-versus-host disease (GVHD), and less subsequent chronic GVHD, as compared with G-PB.[1,2] Moreover, G-BM transplantations produce even less chronic GVHD than do steady-state bone marrow grafts.[2] In this study, we report on the immunological cells and the type1/type2 cytokine profile of lymphocytes present in G-PB and G-BM harvests.

The donors, consisting of eight men and seven women, provided informed consent and ranged in age from 13 to 65 years, with a median age of 40 years. Approval for this study was obtained from the Institutional Review Board and Ethical Committee of the Health Center at Peking University. Samples of G-BM and G-PB were obtained, isolated, and evaluated as described previously.[3,4] It was ensured that the G-BM and G-PB had the same cell concentration. Statistical comparisons were performed using t-tests for independent samples. The lymphocyte proliferation ability in G-PB (stimulation index: 1.13±0.24) was significantly higher than in G-BM (0.98±0.14, p=0.045; n=15 experiments). This finding suggests hyporesponsiveness of T cells in G-BM and is likely related to the lower incidence of GVHD observed in G-BM transplants.

The quantities of interferon-γ (IFN-γ) (13.19±14.33 pg) and interleukin-4 (IL-4) (3.67±1.77 pg) secreted per microliter of G-PB mononuclear cells were, respectively, 8.5- and 4.5-fold higher than those of G-BM mononuclear cells (1.31±0.57 pg and 0.75±0.24 pg; p<0.001) (Figure 1). The ratio of IL-4/IFN-γ was significantly lower in G-PB than in G-BM (0.33±0.23 vs. 0.73±0.16, p<0.001). These results suggest that bone marrow T cells could be easily polarized from Th1 to Th2 and that patients transplanted with G-PB could accept more type1/type 2 cytokines than could patients transplanted with G-BM. The type-1 to type-2 immune deviation after *in vivo* application of G-CSF is associated with decreased acute GVHD or with the development of a *chronic* GVHD syndrome, characterized by decreased mortality and autoantibody formation.[5]

Krenger and Ferrara have proposed a model in which type 1 cytokines (IL-2, IFN-γ) are involved in the physiopathology of acute GVHD, and type 2 cytokines (IL-4, IL-10) play a crucial role in the physiopathology of chronic GVHD.[5] Fowler *et al*. found that type 1 and type 2 cells appear to play different roles in mediating GVHD and graft-versus-leukemia (GVL) effects.[6,7] Furthermore, type 2 T cells are more resistant to CD95 (Fas)-dependent activation-induced cell death than are type 1 T cells.[8] Therefore, the high quantities of type 1 and type 2 cytokines in G-PB may be related to the different outcomes of the GVL effect and GVHD after G-PB and G-BM transplantation.[1,2]

The quantities of nucleated cells and monocytes in G-PB were, respectively, 4- and 43-fold higher than in G-BM harvests (p<0.001), all lymphocyte subsets exhibited 26- to 46-fold higher cell counts (p<0.001), and the CD4/CD8 ratio was also significantly higher in G-PB than in G-BM (1.59±0.53 vs. 0.91±0.29, p<0.001). These findings indicate that patients transplanted with G-PB may accept more T cells and monocytes than patients transplanted with G-BM (*clinical data not shown*). The cell counts of dendritic cell (DC) 1 and DC2 subgroups in G-PB were, respectively, 11- and 7-fold higher than those in G-BM (p<0.001 and