10) among patients with a MPL >2 days ($p$=0.3).

Most studies point to elevated thrombopoietin levels and low levels of glycocalicin in the case of a hypomegakaryocytic thrombocytopenia.[2] However, in our study a reduced PPR was not associated with elevated thrombopoietin levels, but rather with a high glycocalicin-index. Comparable results were recently obtained in myelodysplastic patients.[6] These data suggest that the reduced PPR is not due to a decline in the mass of Mpl-bearing cells in the bone marrow, but indicate an increased release of GP-Ibα complex in the circulation. This might be a consequence of shedding of the receptor complex from destroyed megakaryocytes and/or platelets in the bone marrow. This is also in concordance with the lack of significant correlation observed between the glycocalicin index and MPL, suggesting that the platelet antibody not only affects platelets but also megakaryocytes.[7,8]

The finding of a lower PPR in patients with more pronounced thrombocytopenia suggests more prominent intramedullary destruction in more severe ITP. In conclusion, the present study indicates that (i) that thrombopoietin levels and the glycocalicin index are related to the dynamics of megakaryocyte and platelet kinetics in bone marrow and peripheral blood; (ii) in ITP patients with a decreased PPR, increased platelet and/or megakaryocyte destruction might occur in the bone marrow, as recently demonstrated by ultrastructural studies.[9] Further research is needed to evaluate the usefulness of determining the glycocalicin index in ITP in clinical practice.

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*Thrombosis*

## Risk of thromboembolism in patients with idiopathic autoimmune hemolytic disease and antiphospholipid antibodies: results from a prospective, case-control study

**During a period of 4 years, 21 consecutive patients with newly diagnosed idiopathic autoimmune hemolytic disease (IAHD) and 42 healthy, sex- and age-matched subjects, were tested for the presence of antipospholipid antibodies (APA). At diagnosis, APA were detected in 10/21 (47.6%) patients and in 2/42 (4.76%) controls ($p$< 0.01). No thromboembolic events were registered during the follow-up period.**

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From January 1996 to January 2000, 47 patients aged > 20 years were diagnosed with autoimmune hemolytic disease (AHD) at the Hematology Department of the University "La Sapienza" of Rome. Of these 47 consecutive patients, 26 had secondary AHD (SAHD) and 21 had idiopathic AHD (IAHD). The main characteristics of the IAHD patients at diagnosis are reported in Table 1. All these patients were tested for antiphospholipid antibodies (lupus anticoagulant, anticardiolipin and anti-β2-glycoprotein I antibodies) at diagnosis, after 8 to 10 weeks and then every six months or when clinically required. We did not test the anti-phospho-ethanolamine antibodies usually associated with IAHD. The results were then compared to those obtained in 42 healthy, sex- and age-matched subjects who tested negative to a panel of several auto-antibodies. Controls were tested at the entry into the study and when APA-positive, after 8-10 weeks for confirmation. Lupus anticoagulant (LAC) was detected according to the criteria indicated by the ISHT,[1] utilizing two procedures: the kaolin clotting time (KCT) and the diluted Russel's viper venom time (DRVVT). Tested plasmas were considered LAC positive only if at least one of the two procedures was diagnostic for LAC. Anticardiolipin antibodies (ACA) of Ig-G type (ACA-IgG) and anti-β2-glycoprotein I (anti-β2 GPI) antibodies were measured with standardized ELISA assays. The differences between the variables, were calculated by the $\chi^2$ test, using a 2×2 table. Differences were considered statistically significant when $p$< 0.05 (two tailed).

Overall, at diagnosis, 10/21 (47.6%) IAHD patients and

**Table 1.** Clinical and laboratory features of the IAHD patients.

| | Patient | Sex | Age (years) | IAHD type | Hb g/dL | Reticulocytes (×10⁶/L) | LDH (U/L) | Tolal bilirubin (mg/dL) | Therapy |
|---|---|---|---|---|---|---|---|---|---|
| < 60 years | | | | | | | | | |
| | 1 | M | 25 | IgG | 12.2 | 110 | 450 | 2 | NO |
| | 2 | M | 34 | IgM | 15 | 90 | 230 | 1.8 | NO |
| | 3 | M | 36 | IgG | 12 | 100 | 300 | 1.9 | NO |
| | 4 | F | 36 | D-L | 14 | 115 | 290 | 2 | NO |
| | 5 | M | 50 | IgG | 7.1 | 130 | 850 | 5 | PDN+AZA |
| | 6 | M | 52 | IgM | 11 | 100 | 580 | 2.4 | PDN |
| | 7 | M | 53 | IgG | 5.8 | 127 | 900 | 5.5 | PDN |
| | 8 | F | 48 | IgG | 9.3 | 118 | 870 | 4.1 | PDN+AZA |
| | 9 | F | 52 | IgG | 11 | 113 | 400 | 2.1 | PDN |
| | 0 | F | 59 | IgG | 6 | 113 | 980 | 5.7 | PDN+AZA |
| | 11 | F | 59 | IgG | 5.6 | 121 | 978 | 4.9 | PDN+AZA |
| | 12 | F | 59 | IgG | 9.6 | 126 | 600 | 3.6 | PDN+AZA |
| ≥ 60 years | | | | | | | | | |
| | 13 | M | 62 | IgM | 14 | 99 | 290 | 1.6 | NO |
| | 14 | M | 63 | IgG | 8.6 | 114 | 860 | 3.9 | PDN |
| | 15 | M | 78 | IgM | 12 | 120 | 300 | 2 | NO |
| | 16 | F | 61 | IgM | 12.8 | 120 | 370 | 2.4 | NO |
| | 17 | F | 69 | IgM | 12 | 100 | 280 | 1.9 | NO |
| | 18 | F | 72 | IgM | 12 | 98 | 300 | 2.2 | NO |
| | 19 | F | 75 | IgM | 5 | 120 | 980 | 4 | PDN |
| | 20 | F | 76 | IgM | 12 | 150 | 300 | 1.6 | NO |
| | 21 | F | 79 | IgG/IgM | 9.9 | 135 | 500 | 4.3 | PDN |

*PDN: prednisone at dosages of 0.5-1.5 mg/kg/day; AZA: azathioprine; D-L: Donath-Landsteiner hemolysin.*

**Table 2.** APA in IAHD patients and the control group.

| Apa | IAHD patients | Control group | p |
|---|---|---|---|
| LAC[a] | 4/21 | 1/42 | **0.01** |
| ACA (IgG[b] + Antiβ2GPI[c]) | 4/21 | 1/42 | **0.01** |
| LAC+ Antiβ2GPI | 2/21 | 0/42 | n.s. |
| **Total APA positive patients** | **10/21** | **2/42** | **0.01** |

[a]*LAC was present if KCT ratio > 1.30 and/or DRVVT ratio >1.20;*
[b]*ACA IgG ≥ 20;* [c]*Antiβ2GPI ≥ 10.*

2/42 (4,76%) controls had pathological values of APA ($p<0.01$); the detailed results of the APA assays are presented in Table 2. Of the 10 APA positive patients 5 (50%) required treatment for IAHD and 3/5 (60%) obtained complete remission (CR). In contrast, 6/11 (54.5%) treated patients for IAHD were APA negative and 5/6 (83.3%) achieved CR. The difference in CR rate between APA positive and negative patients was not statistically significant. Among the 8 CR patients, 4 (50%) relapsed, of these 2 were APA positive and 2 negative. At time of relapse, the 2 APA negative patients converted to being APA positive; however, positivity was not confirmed after 8 weeks. One patient affected by cold IAHD, who was APA negative at diagnosis, experienced two transient ischemic attacks (TIA). During the follow-up, 6/10 (60%) APA positive patients became negative and, of these, 3 (50%) had been treated for IAHD.The remaining 4/10 (40%) APA positive patients, of whom 2 had been treated for IAHD, remained persistently APA-positive after 36, 44, 54 and 84 months. After a mean follow-up of 55 months (range 36-84), similar to that of the patients described by Pullarkat *et al.*,[2] none of the APA-positive patients at diagnosis or at the last follow-up had experienced thromboembolic events.

This prospective study indicates that APA are statistically more frequent in newly diagnosed IAHD patients than in normal controls and, according to literature, the steroid treatment does not modify the LAC results or ACA-IgG and anti-β2 GPI levels, even in patients who achieve CR. As for the thromboembolic risk in these APA-positive IAHD patients, none experienced a thromboembolic event. Only one patient, 78 years old, affected by cold IAHD and not receiving therapy, developed TIA despite being APA-negative at diagnosis. In contrast, a 59-year old female affected by warm IAHD requiring treatment with prednisone and azathioprine did not have any thromboembolic events, despite persistently high levels of KCT, DRVVT, and anti-β2 GPI and the use of estrogens. Our data did not confirm that the presence of APA in IAHD is associated with an increased risk of thromboembolism as recently demonstrated by Pullarkat *et al.*[2] in patients with autoimmune hemolytic anemia after a similar follow-up. This behavior is different from that reported in patients with systemic lupus erythematosus and SAHD, who frequently develop an APA syndrome,[3,4] probably enhanced by the involvement of the endothelium, and the production of inflammatory cytokines. In conclusion, unlike Pullarkat *et al.*,[2] we failed to reveal any association between APA and development of thromboembolism in patients with IAHD. However, given the low number of patients with APA and the short follow-up period these negative results should be considered with caution before a higher risk of thromboem-

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.

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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%).*