Anti-phospholipid antibodies are a heterogeneous group of antibodies that include lupus anticoagulant (LA), anticardiolipin (aCL) antibodies, anti-β2 glycoprotein-I (aβ2-GPI) antibodies, and antiprothrombin (aPT) antibodies (1-4). aPT antibodies were first described in 1959 by Loelinger in patients who were carriers of antiphospholipid antibodies (5), and then in other clinical conditions (6). While it has been clearly demonstrated that LA and, to a lesser extent, aCL antibodies represent a risk factor for venous thromboembolism (VTE) (7-12), the role of aβ2-GPI antibodies, and that of aPT antibodies is still undefined (13-15). Although a few recent studies have advocated a potential role of aPT antibodies in the development of VTE in patients with or without systemic lupus erythematosus (16-19), the question still remains controversial (15, 20).

We investigated the presence of antiphospholipid antibodies in a wide series of consecutive patients with acute VTE, as confirmed by objective tests. The aim of the current investigation was to evaluate the prevalence of aPT antibodies, and to assess its association with the presence of prior thromboembolism.

Methods

Patients

All consecutive patients, who referred to the Thrombosis Unit of Padua University between January 2001 and October 2002...
for an episode of acute VTE, were eligible for the investigation. Patients with systemic lupus erythematosus, lupus-like disease or cancer were excluded, as were pregnant women and carriers of antithrombin, protein C or S defect, factor V Leiden and prothrombin gene mutation. All patients gave a careful history, outlining the presence of risk factors for venous thrombosis with special attention to past history of VTE prior to laboratory determination. A reported venous thromboembolic event was accepted as such, if it had been confirmed by objective testing (21-23).

After recording patient history, blood samples were collected into plastic syringes containing 3.8% (wt/vol) sodium citrate in a ratio of 0.1:0.9 (vol/vol) anticoagulant to blood. Platelet poor plasma (PPP) was prepared by centrifugation at 2000 g for 10 min and re-centrifugation of the harvested plasma under the same conditions. Aliquots of PPP were stored at -70°C until use.

**Laboratory determinations**

**Anti-prothrombin antibodies**

aPT antibodies IgG and IgM were assessed using human anti-Prothrombin IgG/IgM supplied by Orgentec Diagnostica GmbH, Mainz, Germany. These are enzyme-linked immunoeassay kits for IgG and for IgM, in which high purified prothrombin is used for coating the strips. IgG and IgM class anti-prothrombin antibodies reference were used to perform an assay calibration and to carry out the IgG and IgM evaluation. The calibration curves were tested in duplicate. Results were plotted on log-log graph paper, and the IgG and IgM level of the patient’s plasma read from the calibration curve. A normal range was established and levels >10 GPl/units/ml were considered abnormal for IgG while levels >8 for IgM. This cut-off is approximately two standard deviations greater than the mean of the control subjects. Each test was performed in duplicate.

**Other antiphospholipid antibodies**

For the detection of LA, the guidelines recommended by the Subcommittee for Standardization of the International Society on Thrombosis and Haemostasis were followed as reported elsewhere (24). Both activated partial thromboplastin time (aPTT) using a lupus anticoagulant-sensitive reagent (PTT-La kit, Stago-Boehringer, Mannheim, Milan, Italy) and dilute Russell viper venom time (dRVVT) (LA1 screening reagent, Dade-Behring, Marburg, Germany) were performed. The prevalence of antiphospholipid antibodies, as well as that of aPT, aCL, and aβ2-GPI antibodies among all patients with VTE was calculated according to standard methods. The association between each category of antiphospholipid antibodies, and the prevalence of previous VTE was calculated and expressed as odds ratio (OR) and its 95% confidence intervals (CI). Next, a multivariate analysis was performed, including in the system age (older than 65 vs younger than 65), sex, and the modality of clinical presentation (idiopathic vs secondary to acquired risk factors). The association between each category of antiphospholipid antibodies and the prevalence of previous VTE was recalculated and expressed as OR and 95% CI. Calculations were performed using SPSS 11.5.1 statistical package.

**Results**

Out of 278 eligible patients, 42 were excluded because of hereditary thrombophilia (22), cancer (10), pregnancy (7) or systemic dRVVT in the presence of a normal aPTT, then dRVVT –based confirmatory tests (LA2 confirmation reagent, Dade-Behring, Marburg, Germany) were performed.

aCL antibodies were assessed using Anti-Cardiolipin IgG/IgM supplied by Orgentec Diagnostica GmbH, Mainz, Germany. These are enzyme linked immunoassay kits for IgG and for IgM in which purified cardiolipin saturated with human β-2 Glycoprotein I is used for coating the strips. IgG and IgM class anti-cardiolipin antibodies reference were used to perform an assay calibration, and to carry out the IgG and IgM evaluation. The calibration curves were tested in duplicate. Results were plotted on log-log graph paper and the IgG and IgM level of the patient’s plasma read from the calibration curve. A normal range was established and level >10 GPl/units/ml were considered abnormal for IgG while level >8 for IgM. This cut-off is approximately two standard deviations greater than the mean of the control subjects. Each test was performed in duplicate.

αβ2-GPI antibodies were assessed using anti-β2-Glycoprotein I IgG/IgM kit supplied by Orgentec Diagnostica GmbH, Mainz, Germany. These are enzyme linked immunoassay kits for IgG and for IgM in which purified β2-GPI is used for coating the strips. β2-GPI IgG and β2-GPI IgM reference were used to perform an assay calibration and to carry out the αβ2-GPI evaluation. The calibration curves were tested in duplicate. Results were plotted on log-log graph paper and the αβ2-GPI level of the patient’s plasma read from the calibration curve. A normal range was established and level >10 GPl/units/ml were considered abnormal. This cut-off is approximately two standard deviations greater than the mean of the control subjects. Each test was performed in duplicate.
lupus erythematosus (3). Therefore, 236 patients with acute VTE were enrolled in the current investigation, of whom 51 (21.6%) had pulmonary embolism only. Laboratory determinations showed the presence of antiphospholipid antibodies in 85 patients (36.0%), of whom 24 (28.2%) were carriers of aPT antibodies, isolated in 7 and associated with other categories of antiphospholipid antibodies in 17. Thus, the presence of aPT antibodies (24/236) was identified in 10.2% of the entire cohort. The main demographic and clinical characteristics of study patients, including the prevalence of potential risk factors for venous thrombosis, are shown in Table 1, separately for patients with and without antiphospholipid antibodies. No significant differences were observed for any of the considered items.

A history of a previous thromboembolism was identified in 56 of the 236 patients (23.7%), and was significantly more frequent in carriers (29/85, 34.1%), than in non-carriers of antiphospholipid antibodies in 85 patients (36.0%), of whom 24 (28.2%) were carriers of aPT antibodies, isolated in 7 and associated with other categories of antiphospholipid antibodies in 17. Thus, the presence of aPT antibodies (24/236) was identified in 10.2% of the entire cohort. The main demographic and clinical characteristics of study patients, including the prevalence of potential risk factors for venous thrombosis, are shown in Table 1, separately for patients with and without antiphospholipid antibodies. No significant differences were observed for any of the considered items.

A history of a previous thromboembolism was identified in 56 of the 236 patients (23.7%), and was significantly more frequent in carriers (29/85, 34.1%), than in non-carriers of antiphospholipid antibodies (27/151, 17.8%; OR = 2.4; 95% CI, 1.3 to 4.4) (Table 2). The prevalence of prior thromboembolism among patients with aPT antibodies (12 of 24, 50.0%), LA (22 of 54, 40.7%), aCL antibodies (13 of 35, 31%) and αβ2-GPI antibodies (11 of 27, 40.7%) was significantly higher than that observed in patients without antiphospholipid antibodies (Table 2). It is of interest that, of the 7 patients who were identified as carriers of isolated aPT antibodies a history of previous thromboembolism was identified in 3.

When a multivariate analysis was performed, including in the system age, sex, and modality of clinical presentation, aPT antibodies were still independently associated with the presence of prior thromboembolism (OR = 3.3; 95% CI, 1.3 to 8.6), whereas the other categories of antiphospholipid antibodies were not (Table 2). As expected, idiopathic presentation was also independently associated with the presence of multiple episodes of VTE (OR = 6.3; 95% CI, 3.2 to 12.2).

### Discussion

In contrast with the extensive documentation on the risk of thromboembolic disorders in carriers of LA and, to a lesser extent, aCL antibodies, the thrombotic potential of αβ2-GPI antibodies and aPT antibodies is less well defined (13-20). While high levels of aPT antibodies have been found to be an independent risk factor of VTE (17, 25), other reports failed to correlate these antibodies with the occurrence of thromboembolic disorders (15, 20). It should be noted, however, that available studies have important methodological differences, making it difficult to compare their results. The large majority of available studies have indeed a retrospective design, and lack an appropriate control group. Contributing to this uncertainty is the difficulty in identifying a group of patients, who are positive for aPT antibodies, but negative for the other categories of antiphospholipid antibodies.

The results of our study suggest that aPT antibodies might play a major role in the development of recurrent VTE in patients with venous thrombosis. According to the results of a multivariate analysis, aPT antibodies were indeed found to be independently associated with a history of previous thromboembolism, a feature that was only shared by patients who referred with idiopathic thrombosis. It is interesting to note that, in our study we were able to identify a subgroup of 7 patients with isolated aPT antibodies while preserving a high rate of recurrent thromboembolism (3 of 7).

Care was taken that bias did not influence the results of our study. We considered consecutive unselected patients with an acute episode of VTE that was objectively confirmed. None of these patients had confounding associated immune diseases such as systemic lupus erythematosus. A priori defined stringent criteria were used to diagnose a previous VTE episode. Finally, the determination of aPT antibodies was performed according to widely accepted guidelines.

What are the practical implications of our results? It is widely accepted that, in order to make therapeutic decisions in patients referring with an episode of VTE, it is crucial to identify determinants of the recurrence risk. Although we do not know with certainty the initial antibody status at the time of the index VTE episode in our patients, we suspect that aPT antibod-

### Table 1: Main demographic and clinical features of patients with and without antiphospholipid antibodies.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>aPL + (n=85)</th>
<th>aPL - (n=151)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (median, range)</td>
<td>57 (19-81)</td>
<td>62 (21-87)</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>52:33</td>
<td>96:55</td>
</tr>
<tr>
<td>Risk factors of venous thrombosis:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- none (idiopathic)</td>
<td>48 (56.5%)</td>
<td>80 (53.0%)</td>
</tr>
<tr>
<td>- recent trauma/fracture</td>
<td>13 (15.3%)</td>
<td>26 (17.2%)</td>
</tr>
<tr>
<td>- recent surgery (&lt; 3 months)</td>
<td>10 (11.8%)</td>
<td>20 (13.2%)</td>
</tr>
<tr>
<td>- prolonged immobilization (&gt; 7 days)</td>
<td>9 (10.6%)</td>
<td>17 (11.3%)</td>
</tr>
<tr>
<td>- estrogen therapy</td>
<td>5 (8.8%)</td>
<td>8 (5.3%)</td>
</tr>
<tr>
<td>Modality of clinical presentation:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DVT with or without pulmonary embolism</td>
<td>70 (82.3%)</td>
<td>115 (76.1%)</td>
</tr>
<tr>
<td>Pulmonary embolism only</td>
<td>15 (17.7%)</td>
<td>36 (23.9%)</td>
</tr>
</tbody>
</table>

### Table 2: Strength of the association of each category of antiphospholipid antibodies with prior thromboembolism.

<table>
<thead>
<tr>
<th>Category</th>
<th>No of patients</th>
<th>Prior VTE</th>
<th>Univariate analysis OR (95% CI)</th>
<th>Multivariate analysis OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>236</td>
<td>56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aPT</td>
<td>24</td>
<td>12</td>
<td>3.8 (1.6-9.1)</td>
<td>3.3 (1.3-8.6)</td>
</tr>
<tr>
<td>LA</td>
<td>54</td>
<td>22</td>
<td>2.9 (1.5-5.8)</td>
<td>1.8 (0.8-4.0)</td>
</tr>
<tr>
<td>aCL</td>
<td>35</td>
<td>13</td>
<td>2.1 (1.0-4.7)</td>
<td>1.2 (0.4-3.0)</td>
</tr>
<tr>
<td>αβ2-GPI</td>
<td>27</td>
<td>11</td>
<td>2.3 (1.1-5.8)</td>
<td>1.0 (0.3-3.3)</td>
</tr>
</tbody>
</table>
ies were already present. If this is the case, the detection of these antibodies should be regarded as a predictor of VTE recurrences. According to the results of a multivariate analysis including patients’ age and modality of clinical presentation, multiple thrombotic episodes were indeed experienced by carriers of these antibodies three times as frequently as they were by non-carriers. Consequently, whenever the assessment of a thrombophilic status is deemed appropriate in a patient with venous thromboembolism, the search for aPT antibodies might be added to the array of the most commonly investigated thrombophilic abnormalities.

In conclusion, the search for aPT antibodies has the potential to identify a subgroup of patients at higher risk of multiple VTE episodes. Further prospective studies are needed to better define the role of aPT antibodies in the development of (recurrent) thromboembolism, as well as to investigate the benefit-to-risk of tailoring the duration of anticoagulation according to the presence of these antibodies.

References