Lupus (2012) 21, 682–687
http://lup.sagepub.com

LUPUS AROUND THE WORLD

Clinical implications and prognostic significance of thrombocytopenia in Tunisian patients with systemic lupus erythematosus

M Jallouli[1], M Frigui[1], S Marzouk[1], M Snoussi[1], M Kechaou[1], N Kaddour[1], H Masmoudi[2], M Elloumi[3] and Z Bahloul[1]

[1]Department of Internal Medicine, Hedi Chaker University Hospital, Sfax, Tunisia; [2]Department of Immunology, Habib Bourguiba University Hospital, Sfax, Tunisia; and [3]Department of Hematology, Hedi Chaker University Hospital, Sfax, Tunisia

Objective: The objective of this study was to determine the role of thrombocytopenia in terms of disease manifestations, disease activity and prognostic impact in a cohort of Tunisian systemic lupus erythematosus (SLE) patients. **Methods:** The charts of 182 SLE patients diagnosed between 1996 and 2009 were retrospectively reviewed. The clinical manifestations, immunological profiles, disease activity, SLE relapses and survival rate at the time of follow-up were recorded. **Results:** Thrombocytopenia (<100,000/mm^3) and severe thrombocytopenia (<20,000/mm^3) was observed in 19.2% and 4.4%, respectively. Hemorrhagic manifestations were observed in 11 patients (31.4%). Thrombocytopenia was significantly associated with splenomegaly, renal disorders, neurologic manifestations, arterial thrombosis, leucopenia, low C3 level at SLE diagnosis, SLE relapses and infectious complications. Using multivariate logistic regression, thrombocytopenia was independently associated with splenomegaly (odds ratio [OR] = 9.36, $p = 0.001$), neurologic manifestations (OR = 4.6, $p = 0.006$) and renal disease (OR = 4.15, $p = 0.02$). By multivariable Cox proportional hazard regression analyses, thrombocytopenia was associated with the occurrence of mortality after adjusting for variables known to influence it (hazard ratio [HR] = 1.79, $p = 0.045$). The cause of death was unrelated to hemorrhagic complications in all patients. **Conclusion:** Our results, concerning North-African SLE patients, confirm the findings of previous studies which suggest that thrombocytopenia correlates with more severe disease and has a negative impact on the survival of lupus patients. *Lupus* (2012) **21,** 682–687.

Key words: Systemic lupus erythematosus, Tunisia, thrombocytopenia, clinical implication, prognosis

Introduction

Thrombocytopenia is a common clinical manifestation and one of the hematological criteria of systemic lupus erythematosus (SLE) according to the American College of Rheumatology (ACR) classification criteria.[1] Its reported prevalence in large series of SLE patients ranges from 10% to 25%.[2] In addition serving as a useful diagnostic criterion for SLE, thrombocytopenia is associated, according to several studies, with neuropsychiatric manifestations,[3–6] hemolytic anemia,[7] antiphospholipid syndrome,[8] kidney disease,[3,6,7,9,10] and death.[3,5,9,11,12] These studies have involved White SLE patients, Hispanics, Asians, and African Americans, but not North Africans.

The objective of this study was to ascertain the role of thrombocytopenia in relation to disease manifestations, activity and prognosis in a cohort of Tunisian SLE patients.

Patients and methods

We retrospectively reviewed the charts of SLE patients who attended the Department of Internal Medicine at the Hédi Chaker University

Correspondence to: Moez Jallouli, Department of Internal Medicine, Hedi Chaker University Hospital, Sfax, Tunisia
Email: jallouli5moez@yahoo.fr
Received 11 July 2011; accepted 17 January 2012

10.1177/0961203312438630

Hospital, Sfax, Tunisia, from January 1996 to December 2009. All patients fulfilled four or more criteria for the diagnosis of SLE as defined by the ACR.[1]

In general, patients were seen at 4-month intervals. During each visit, the patient's history was reviewed and a physical examination and routine laboratory tests performed. Data on demographic characteristics, clinical and laboratory manifestations, and types of treatment received during these events, as well as all comorbidities, were collected and recorded on standard forms. The platelet count at the onset of thrombocytopenia, hemorrhagic manifestations, and the number of relapses were also recorded. Thrombocytopenia secondary to lupus (but not to other causes) was defined according to the ACR criteria as a platelet count of 100×10^9/l. We defined thrombocytopenia as severe when the platelet count was $<20 \times 10^9$/l, moderate between 20 and 50×10^9/l, and mild at $>50 \times 10^9$/l.

A relapse of thrombocytopenia was defined as a reduction in platelet count below the threshold of 100,000/mm^3 after at least two consecutive normal platelet counts (increase to >100,000/mm^3).

The principal clinical manifestations were defined as described by the American Rheumatism Association glossary committee.[13] Splenomegaly was diagnosed by clinical examination or abdominal Doppler ultrasound. Antinuclear antibodies (ANA) were evaluated by the indirect immunofluorescence technique with either mouse liver or HEp-2 cell substrate (Alphadia, Waver, Belgium). Anti–DNA antibodies were assayed by indirect immunofluorescence against *Crithidia luciliae* (Binding Site, Birmingham, United Kingdom) and by enzyme-linked immunosorbent assay if the immunofluorescence results were negative. Anti-extractable nuclear antigen antibodies (anti-Sm, anti-SSA, anti-SSB and anti-RNP) were analyzed by the Euroline ANA-profile 1 (Euroimmun, Lübeck, Germany). Anti-nucleosome antibodies were detected by immunodot. Complement and rheumatoid factor were assessed by nephelometry, and IgG and IgM anticardiolipin antibodies (aCL) by enzyme-linked immunosorbent assay (Binding Site, Birmingham, United Kingdom). Lupus anticoagulant was detected using dilute Russell Viper Venom Time (dRVVT), which screens for lupus-like anticoagulant, and DVV Confirm (ratio) which confirms lupus anticoagulant presence in plasma.

Disease activity at diagnosis was measured by the SLE Disease Activity Index (SLEDAI).[14] However, thrombocytopenia is one of the parameters included in the SLEDAI; we have therefore excluded it from the SLEDAI score and report a modified SLEDAI score.

Statistical analysis

Standard χ^2 and Fisher's exact tests were used to analyze qualitative differences, and Student's *t*-test to compare the means in large independent samples of similar variance. A *p*-value <0.05 was defined as statistically significant. When several independent variables appeared significant in the univariate analysis, a logistic regression test was performed for multivariate analysis to rule out possible confounding variables. In this case, only those variables statistically significant in the multivariate analysis were considered significant results. Odds ratios (ORs) were calculated to assess the risk of each variable.

The contribution of thrombocytopenia to mortality was examined by a multivariable Cox proportional hazard regression model, adjusting for variables previously found to be associated with mortality (age,[5,15,16] sex,[15–17] race,[5,18] disease activity at baseline,[19] neurologic manifestations,[16, 20] kidney disease,[15,20] and high-dose steroid treatment[11]). The statistical analysis was performed with SPSS/PC 13.0 software.

Results

The study included 182 patients, 160 (87.9%) women and 22 (12.1%) men (for a sex ratio of 7.3 to 1). The mean age at SLE diagnosis was 30.6 ± 10.9 years. The mean duration of follow-up was 4.6 ± 5 years. At the time of analysis, 11 patients (6%) had died and 31 (17%) had been lost to follow-up. Thrombocytopenia was observed in 35 (19.2%) patients.

Characteristics of thrombocytopenia

Thrombocytopenia was one of the initial manifestations of SLE in 28 of the 35 (80%) patients with

Table 1 Severity of hemorrhagic manifestations in patients with thrombocytopenia

Hemorrhagic events	*Absent N (%)*	*Minor N (%)*	*Major N (%)*	*Total N (%)*
Platelet level (mm^{-3})				
50,000–100,000	20 (83.3)	4 (57.1)	1 (25)	25 (71)
20,000–50,000	1 (4.2)	1 (14.3)	0	2 (6)
<20,000	3 (12.5)	2 (28.6)	3 (75)	8 (23)
Total *N* (%)	24	7	4	35 (100)

platelet deficiency. In the remaining 7 (20%) patients, it followed the SLE diagnosis by a median period of 60 months (range 18–300).

The median platelet count at the first episode of thrombocytopenia was 75,500/mm^3 (range 5000–100,000). Thrombocytopenia was severe in 8 patients (23%), moderate in 2 (6%) and mild in 25 (71%). Hemorrhagic manifestations were observed in 11 (31.4%) (Table 1). Major hemorrhagic events included diffuse ecchymosis (three cases) and ocular hemorrhage (one case). Purpura, gum bleeding, and mild epistaxis were considered minor and were observed in seven cases. At the onset of thrombocytopenia, the platelet count was strongly associated with hemorrhagic manifestations ($p = 0.002$).

A bone marrow aspiration was performed during thrombocytopenic episodes in 20 cases. Megakaryocytes, normal or increased in all specimens, reflected peripheral platelet destruction.

Case–control analysis

Table 2 summarizes the individual demographic data, cumulative clinical manifestations, serologic features, and pharmacologic treatment of patients with thrombocytopenia and those without it. In the univariable analysis, thrombocytopenia was positively associated with splenomegaly ($p < 0.001$), kidney disorders ($p < 0.001$), neurologic manifestations ($p = 0.008$), arterial thrombosis ($p = 0.013$) and leukopenia ($p = 0.012$). A low C3 level at SLE diagnosis was more frequent in patients with thrombocytopenia than in those without it ($p = 0.008$). Similarly, the proportion of patients who had SLE relapses (60% versus 30.6%; $p = 0.001$) and infectious complications (62.9% versus 36.1%; $p = 0.004$) was higher among those with thrombocytopenia than among those without.

Multivariate logistic regression found that thrombocytopenia was independently associated with splenomegaly (OR 9.36, 95% confidence interval [CI] 2.37–36.89, $p = 0.001$), neurologic manifestations (OR 4.6, 95% CI 1.54–13.72, $p = 0.006$) and kidney disease (OR 4.15, 95% CI 1.24–13.82, $p = 0.02$).

The multivariable Cox proportional hazard regression analyses showed that thrombocytopenia was associated with mortality after adjustment for variables known to influence it (hazard ratio [HR] 1.79, 95% CI 1.01–3.17, $p = 0.045$). Figure 1 shows the survival curves of our patients with and without thrombocytopenia.

Table 2 Cumulative clinical manifestations and serologic features on last visit according to presence of thrombocytopenia

	SLE patients		
	With thrombocytopenia, n = 35	*Without thrombocytopenia, n = 147*	*p-value*
Age at SLE diagnosis (years)*	28.8 ± 9.9	31 ± 11.1	ns
Male	5(14.3)	17(11.6)	ns
Manifestations			
Malar rash	19(54.3)	74(50.3)	ns
Discoid rash	2(5.7)	16(10.9)	ns
Photosensitivity	12(34.3)	84(57.1)	0.015
Oral ulcers	10(28.6)	23(15.6)	ns
Arthritis	14(40)	80(54.4)	ns
Pleuritis	9(25.7)	37(25.2)	ns
Pericarditis	13(37.1)	28(19)	0.048
Neurologic	12(34.3)	22(15)	0.008
Seizure	7(20)	16(10.9)	ns
Renal disorder	28(82.9)	73(49.7)	<0.001
Proliferative nephritis	15(42.9)	51(34.7)	ns
Membranous nephritis	2(5.7)	7(4.8)	ns
Leukopenia	24(68.6)	66(44.9)	0.012
Lymphopenia	18(51.4)	78(53.1)	ns
Anemia < 10 g/dl	31(88.6)	101(68.7)	0.018
Arterial thrombosis	4(11.4)	2(1.4)	0.013
Splenomegaly	10(28.6)	5(3.4)	<0.001
Antiphospholipid syndrome	5(14.3)	11(7.5)	ns
Venous thrombosis	0	8	–
Arterial thrombosis	4	1	–
Obstetrical manifestations	1	2	–
Antibody reactivity			
Anti-DNA Abs	25/34(73.5)	95/147(64.6)	ns
Anti-Sm Abs	11/34(32.4)	59/147(40.1)	ns
Anti-SSA Abs	19/34(55.9)	86/147(58.5)	ns
Anti-SSB Abs	11/34(32.4)	30/145(20.7)	ns
Anti-RNP Abs	10/34(29.4)	59/147(40.1)	ns
Anti-nucleosome Abs	14/19(73.7)	58/92(63)	ns
Anticardiolipin Abs	26/30(86.7)	84/118(71.2)	ns
Lupus anticoagulant	10/20(50)	26/82(31.7)	ns
Decreased C3	24/29(82.8)	59/106(55.7)	0.008
Decreased C4	25/29(86.2)	72/105(68.6)	ns
Modified SLEDAI at diagnosis	13.3 ± 5.6	11.8 ± 5.7	ns
Corticosteroids	35(100)	142(96.6)	ns
Antimalarials	30(85.7)	134(91.2)	ns
Cyclophosphamide	9(25.7)	36(24.5)	ns
Infectious complications	22(62.9)	53(36.1)	0.004
SLE relapses	21(60)	45(30.6)	0.001

The percentage values are given in parentheses.
*Values are mean ± SD.
SLE, systemic lupus erythematosus; Abs, antibodies; SLEDAI, SLE Disease Activity Index; ns, not significant.

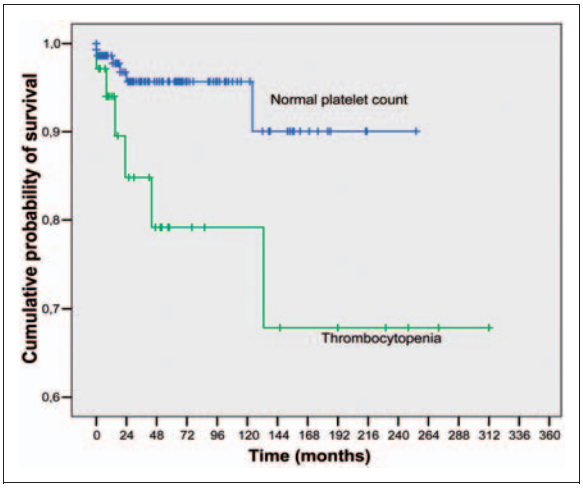

Figure 1 Probability of survival in SLE patients with and without thrombocytopenia.

Thrombocytopenia: treatment and outcome

The duration of follow-up was less than 5 days for two patients. The remaining 33 were treated with corticosteroids, and 30 of them (90.9%) with antimalarials as well. The daily corticosteroid dose was 1 mg/kg in 32 (97%) cases. Eight (24.2%) patients also received cyclophosphamide for proliferative glomerulonephritis or neurologic manifestations. No patient had a splenectomy or intravenous immunoglobulin, and 32 (97%) achieved a satisfactory platelet response (increase to $>100{,}000/mm^3$). Thrombocytopenia persisted in one patient whom we lost sight of for seven months.

Five patients (15.2%) had seven thrombocytopenic relapses during their disease course. Thrombocytopenia was the only activity marker of the disease in only one case.

At the time of these analyses, six (17.1%) patients had died (median 15 months, range 0.1–133 after SLE diagnosis), two of them during thrombocytopenic episodes. No deaths were due to hemorrhagic complications. The main causes of death were active SLE (five cases) and infections (three cases).

Discussion

This longitudinal retrospective study investigated the prevalence of clinical and serological features in a cohort of lupus patients with and without thrombocytopenia. We included only SLE patients who were diagnosed and followed by the same group of specialists in a regional service hospital in Tunisia. Therefore, patients in this cohort were unselected, and the thrombocytopenia data were unlikely to be biased.

We report that 19.2% of patients experienced at least one thrombocytopenic episode after a mean follow-up of 4.6 years. This prevalence is similar to the 20% reported in the USA,[3] 22% in Europe[21] and 25% in Hong Kong.[22]

We found that a significant proportion of patients had their first thrombocytopenic episode at SLE diagnosis or in the first year thereafter. These findings confirm results reported by Sultan et al.[10]

The pathogenesis of thrombocytopenia in SLE is multifactorial. Most studies have recognized the predominant role of antibodies in platelet destruction.[23,24] Other mechanisms proposed to explain this SLE complication include antiphospholipid antibodies, vasculitis, thrombotic microangiopathy, hemophagocytosis, and bone marrow stromal alterations.[25] Our finding that splenomegaly is associated with thrombocytopenia in the multivariate model support the hypothesis that the most common mechanism inducing thrombocytopenia in SLE is the peripheral consumption of platelets.

Other studies have reported, as we do, the association of thrombocytopenia with serious disease manifestations, including neurologic[3–6] and kidney disorders.[3,6,7,9,10] Thrombocytopenia in our patients was also associated with leukopenia and the possibility of predisposition to infectious diseases, which are a well-known major cause of morbidity and mortality in SLE patients.[21,26] These findings are consistent with the results of Zhao et al.[9] The association of thrombocytopenia with lower serum complement C3 levels, generally correlated with disease activity, is consistent with the findings by Ziakas et al.[27] More importantly, we found that thrombocytopenia was an adverse predictor for survival in our SLE population. This too is consistent with the experience of other ethnic groups.[3,5,9,11,12] As other investigators have previously reported,[6,9,28] however, these deaths were rarely a direct result of thrombocytopenia.

The association of thrombocytopenia with arterial thrombosis has not been described by other reports, but it was expected since our four patients with both arterial thrombosis and thrombocytopenia had an associated antiphospholipid syndrome.

In the analysis of autoantibody profiles, there was no significant difference between the two groups, including for the presence of anti-cardiolipin antibodies, which have been widely reported in the literature.[3,10,29]

Most of our patients (97%) responded to treatment with high-dose steroids. This high rate

confirms that steroids remain the treatment of choice in the initial management of thrombocytopenia in SLE.[30,31] The addition of hydroxychloroquine may be an adequate alternative for inducing remission in many patients. Second-line therapeutic agents with azathioprine or cyclophosphamide may be required for patients who fail to respond to steroids or those requiring moderate doses of steroids to maintain their platelet counts. Human normal immunoglobulin has also been used successfully to treat this SLE complication. A number of novel therapeutic approaches to the management of thrombocytopenia, including thrombopoietin receptor agonists and anti-B-cell therapies, in particular, show great promise.[31]

The lack of splenectomies among our patients is indicative of the limited role for this treatment in SLE patients and consistent with the findings of both Ziakas et al.[27] and Zhao et al.[9] who reported splenectomies among only 3.7% and 6.3% of their patients, respectively.

The main limitation of our study is its retrospective nature, which prevents any definitive conclusions about the role of thrombocytopenia in disease manifestations, activity and prognosis. Nor was the number of patients with relapses large enough to allow us to determine the predictors of thrombocytopenic relapse.

In conclusion, thrombocytopenia is a common event in Tunisian SLE patients. Our study of this North-African SLE population confirms the findings of previous studies suggesting that thrombocytopenia in lupus patients is correlated with more severe disease and poorer survival.

Acknowledgement

We would like to express our thanks to Dr Lamia Zaibi, assistant at the University of Manouba, for editing this article.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

1 Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.
2 Keeling DM, Isenberg DA. Haematological manifestations of systemic lupus erythematosus. *Blood Rev* 1993; 7: 199–207.
3 Fernandez M, Alarcon GS, Apte M, *et al*. Systemic lupus erythematosus in a multiethnic US cohort: XLIII. The significance of thrombocytopenia as a prognostic factor. *Arthritis Rheum* 2007; 56: 614–621.
4 Feinglass EJ, Arnett FC, Dorsch CA, Zizic TM, Stevens MB. Neuropsychiatric manifestations of systemic lupus erythematosus: diagnosis, clinical spectrum, and relationship to other features of the disease. *Medicine (Baltimore)* 1976; 55: 323–339.
5 Reveille JD, Bartolucci A, Alarcon GS. Prognosis in systemic lupus erythematosus. Negative impact of increasing age at onset, black race, and thrombocytopenia, as well as causes of death. *Arthritis Rheum* 1990; 33: 37–48.
6 Scofield RH, Bruner GR, Kelly JA, *et al*. Thrombocytopenia identifies a severe familial phenotype of systemic lupus erythematosus and reveals genetic linkages at 1q22 and 11p13. *Blood* 2003; 101: 992–997.
7 Miller MH, Urowitz MB, Gladman DD. The significance of thrombocytopenia in systemic lupus erythematosus. *Arthritis Rheum* 1983; 26: 1181–1186.
8 Drenkard C, Villa AR, Alarcon-Segovia D, Perez-Vazquez ME. Influence of the antiphospholipid syndrome in the survival of patients with systemic lupus erythematosus. *J Rheumatol* 1994; 21: 1067–1072.
9 Zhao H, Li S, Yang R. Thrombocytopenia in patients with systemic lupus erythematosus: significant in the clinical implication and prognosis. *Platelets* 21: 380–385.
10 Sultan SM, Begum S, Isenberg DA. Prevalence, patterns of disease and outcome in patients with systemic lupus erythematosus who develop severe haematological problems. *Rheumatology (Oxford)* 2003; 42: 230–234.
11 Mok CC, Lee KW, Ho CT, Lau CS, Wong RW. A prospective study of survival and prognostic indicators of systemic lupus erythematosus in a southern Chinese population. *Rheumatology (Oxford)* 2000; 39: 399–406.
12 Ward MM, Pyun E, Studenski S. Mortality risks associated with specific clinical manifestations of systemic lupus erythematosus. *Arch Intern Med* 1996; 156: 1337–1344.
13 American Rheumatism Association Glossary Committee. *Dictionary of the rheumatic diseases: signs and symptoms*, Vol. 1, 1st edn. Bayport, NY: Contact Associates International, 1982, p. 1–80.
14 Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992; 35: 630–640.
15 Manger K, Manger B, Repp R, *et al*. Definition of risk factors for death, end stage renal disease, and thromboembolic events in a monocentric cohort of 338 patients with systemic lupus erythematosus. *Ann Rheum Dis* 2002; 61: 1065–1070.
16 Bujan S, Ordi-Ros J, Paredes J, *et al*. Contribution of the initial features of systemic lupus erythematosus to the clinical evolution and survival of a cohort of Mediterranean patients. *Ann Rheum Dis* 2003; 62: 859–865.
17 Alamanos Y, Voulgari PV, Papassava M, Tsamandouraki K, Drosos AA. Survival and mortality rates of systemic lupus erythematosus patients in northwest Greece. Study of a 21-year incidence cohort. *Rheumatology (Oxford)* 2003; 42: 1122–1123.
18 Uribe AG, Alarcon GS. Ethnic disparities in patients with systemic lupus erythematosus. *Curr Rheumatol Rep* 2003; 5: 364–369.
19 Massardo L, Martinez ME, Jacobelli S, Villarroel L, Rosenberg H, Rivero S. Survival of Chilean patients with systemic lupus erythematosus. *Semin Arthritis Rheum* 1994; 24: 1–11.
20 Blanco FJ, Gomez-Reino JJ, de la Mata J, *et al*. Survival analysis of 306 European Spanish patients with systemic lupus erythematosus. *Lupus* 1998; 7: 159–163.
21 Cervera R, Khamashta MA, Font J, *et al*. Morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients. *Medicine (Baltimore)* 2003; 82: 299–308.
22 Mok CC, Lau CS. Lupus in Hong Kong Chinese. *Lupus* 2003; 12: 717–722.

23 Macchi L, Rispal P, Clofent-Sanchez G, *et al*. Anti-platelet antibodies in patients with systemic lupus erythematosus and the primary antiphospholipid antibody syndrome: their relationship with the observed thrombocytopenia. *Br J Haematol* 1997; 98: 336–341.
24 Pujol M, Ribera A, Vilardell M, Ordi J, Feliu E. High prevalence of platelet autoantibodies in patients with systemic lupus erythematosus. *Br J Haematol* 1995; 89: 137–141.
25 Cines DB, Liebman H, Stasi R. Pathobiology of secondary immune thrombocytopenia. *Semin Hematol* 2009; 46: S2–S14.
26 Edwards CJ, Lian TY, Badsha H, Teh CL, Arden N, Chng HH. Hospitalization of individuals with systemic lupus erythematosus: characteristics and predictors of outcome. *Lupus* 2003; 12: 672–676.
27 Ziakas PD, Giannouli S, Zintzaras E, Tzioufas AG, Voulgarelis M. Lupus thrombocytopenia: clinical implications and prognostic significance. *Ann Rheum Dis* 2005; 64: 1366–1369.
28 Ziakas PD, Dafni UG, Giannouli S, Tzioufas AG, Voulgarelis M. Thrombocytopaenia in lupus as a marker of adverse outcome—seeking Ariadne's thread. *Rheumatology (Oxford)* 2006; 45: 1261–1265.
29 Harris EN, Asherson RA, Gharavi AE, Morgan SH, Derue G, Hughes GR. Thrombocytopenia in SLE and related autoimmune disorders: association with anticardiolipin antibody. *Br J Haematol* 1985; 59: 227–230.
30 Arnal C, Piette JC, Leone J, *et al*. Treatment of severe immune thrombocytopenia associated with systemic lupus erythematosus: 59 cases. *J Rheumatol* 2002; 29: 75–83.
31 Hepburn AL, Narat S, Mason JC. The management of peripheral blood cytopenias in systemic lupus erythematosus. *Rheumatology (Oxford)* 2010; 49: 2243–2254.