Association of capillaroscopic microhaemorrhages with clinical and immunological antiphospholipid syndrome

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ABSTRACT

Objective. *Patients with clinical signs of vasculopathy were screened with capillaroscopy for microangiopathy, and its presence was evaluated in the diagnosis of antiphospholipid syndrome (APS). For this purpose, autoantibody profiles in high risk patients with microhaemorrhages were correlated with thrombotic events.*

Methods. *738 patients from a Rheumatology Outpatients cohort were consecutively screened with capillaroscopy. Patients with microhaemorrhages were selected from the total of individuals screened and tested for anticardiolipin (αCL) and anti-beta2 glycoprotein 1 (anti-β2GP1) Abs. Positive autoantibody profile was subsequently correlated with arterial and venous thrombotic events. Patients with scleroderma were excluded from the analysis.*

Results. *149 patients with various rheumatologic conditions and capillary microhaemorrhages were included in the study. Antiphospholipid profile screening in these individuals revealed a 15.4% of newly diagnosed secondary laboratory APS. αCL antibodies and anti-β2-glycoprotein 1 (anti-β2GP1 Abs were both found to independently correlate significantly with thrombotic events. Subanalysis of the type of anti-β2GP1 Abs indicated that the correlation with thrombotic events was significant for IgG-type (p<0.001) and IgM-type (p=0.051), but not IgA-type Abs (p=0.292).*

Conclusion. *In patients with microhaemorrhages αCL and anti-β2GP1 Abs were associated with thrombotic events. The observation that, although IgA type-anti-β2GP1 Abs were detected in patients with microangiopathy, they lacked any significant association with thrombotic complications, suggests, that either the type/conformation of the autoantibodies and/or additional factors may be critical for the development of thromboses. In conclusion, capillaroscopy can aid diagnostically to screen for or verify APS in combination with other parameters.*

Introduction

Nailfold capillaroscopy has proved to be one of the best diagnostic non-invasive imaging techniques to evaluate microcirculation *in vivo* and is increasingly employed in the field of rheumatology (1). Various capillaroscopic patterns have been identified and related to different rheumatologic conditions. In patients with antiphospholipid syndrome (APS), the occurrence of nailfold small-vessel occlusions (thrombotic microangiopathy) and/or alterations in loop diameter have been repeatedly described (2, 3). The most frequent capillaroscopic pattern involves symmetrical microhaemorrhages, which in previous studies was found to relate to the presence of autoantibodies (αCL) (4); it was not however accompanied by functional (rheological) alterations (5). We performed a cross-sectional study to investigate the association of abnormal capillaroscopic findings (microhaemorrhages) with clinical and laboratory (*i.e.* positive antiphospholipid antibody profile) APS, and evaluate the potential of capillaroscopy to aid as a synergistic screening tool in the diagnosis of this syndrome. For this purpose, capillaroscopic findings and matching autoantibody profiling were correlated with the incidence of arterial and venous thrombotic events.

Methods

Seven hundred and thirty-eight patients from a Rheumatology Outpatients cohort were consecutively screened with capillaroscopy (DS Medica, Milan, Italy – 200x magnification). Criteria for selection included clinical signs of, or predisposition for, vasculopathy, *i.e.* Raynaud's phenomenon, livedo reticularis, diagnosis of systemic lupus erythematosus (SLE), positive antiphospholipid profile and/or previous thrombotic events, as well as atypical musculoskeletal symptomatology. Scleroderma patients with the typical dilated loop morphology were excluded (6). All history for thrombotic events was recorded (recurrent miscarriages, cerebrovascular events, arterial thromboses, deep vein thromboses, pulmonary embolism, digital ulcerations, etc.). Patients with evidence of capillaroscopic microangiopathy (microhaemorrhages), not previously screened for antiphospholipid antibodies, were

informed about the finding, and consented to be tested for αCL and anti-β2GP1 Abs. Screening for the presence of these autoantibodies was performed using standard enzyme-linked immunosorbent assays (Varelisa, Phadia GmBH, Munich, Germany). Normal cut-off values were 15U/ml for αCL-G and 6.1U/ml for anti-β2GP1 antibodies. Patients were considered positive when values obtained were >20U/ml and >8.0U/ml respectively. When feasible, positive patients were re-tested at least once after a minimum interval of 1 month.

Statistical analysis was performed using the SPSS 16 software for Windows (SPSS Inc., Chicago, IL, USA). Results were expressed for continuous variables with a normal distribution as mean±standard deviation and with non-normal distributions as median (minimum-maximum). Kolmogorov-Smirnov or Shapiro-Wilk algorithm was used to determine whether each variable had a normal distribution. The Chi-square test with continuity correction or Fisher's exact test – when at least one cell had an expected count of less than 5 – were used to compare frequencies between groups. A probability value of $p<0.05$ was considered statistically significant.

Results

One hundred and forty-nine of the screened patients (85.2%♀ / 14.8%♂, 49.28±14.31 yrs) exhibited capillary microhaemorrhages. Running diagnosis in these patients was undifferentiated connective tissue disease (UCTD) (24.2%), SLE (18.8%), rheumatoid arthritis (RA) (16.1%), primary APS (12.8%), Sjögren's syndrome (4.7%), HLA B51 (3.4%), and vasculitis (2%), Table I. 18/149 patients were positive for αCL, 29/149 for anti-β2GP1 Abs and 7/149 for both. The presence of αCL and anti-β2GP1 Abs were related to history of arterial and/or venous thromboses. Thrombotic events included premature abortions (4 pts), cerebrovascular events (4 pts), pulmonary embolism (4 pts), deep vein thrombosis (3 pts), epileptic crises/ diplopia not attributable to tumours or verified cerebrovascular events (3 pts), digital infarcts/ulcerations (2 pts), arterial thrombosis/

limb ischaemia (1 pt) and coronary events (1 pt). Small vessel thrombotic events such as digital infarcts were included in the arterial thrombotic events category. αCL and anti-β2GP1 Abs were both found to independently significantly correlate with thrombotic events ($p<0.001$). Subanalysis of the type of anti-β2GP1 Abs indicated that the correlation with thrombotic events was significant for G-type ($p<0.001$) and M-type ($p=0.051$-according to the Fisher's test value), but not A-type Abs ($p=0.292$, NS), Table II. No significant association of αCL or anti-β2GP1 Abs was observed in relation to the type of thrombosis (arterial or venous), Table III; however any subgroup analysis re-

garding the type of thrombosis should be interpreted with caution, because of the small number of affected individuals. Antiphospholipid profile testing in patients with microhaemorrhages revealed a 15.4% of newly diagnosed secondary laboratory APS. For the purposes of this study, secondary laboratory APS was defined as the presence of antiphospholipid antibodies in patients with primary rheumatologic conditions other than primary APS in the presence or absence of a positive history of thrombosis. Primary rheumatologic condition in these patients was UCTD (39.1%), SLE (34%), Sjögren's syndrome (8.7%), HLA B51 (8.7%), RA (4.3%) and vasculitis (4.3%). Three patients with a

Table I. Demographics and disease classification of patients with capillaroscopic microhaemorrhages.

	Total	Male	Female
Sex*	149	22	127
Age (yrs)	47.95 ± 14.69	47.92 ± 18.17	47.95 ± 14.32
Duration of disease (yrs)	5 (1-35)	3.5 (2-35)	5 (1-30)
Diagnosis*			
UCTD	36	6	30
SLE	28	5	23
RA	24	4	20
APS	19	0	19
Sjögren	7	0	7
B51	5	1	4
Vasculitis	3	1	2
Other	27	5	22

*values are the number of patients.
UTCD: undifferentiated connective tissue disease; SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; APS: antiphospholipid syndrome.
Other (psoriatic arthritis, undifferentiated arthritis, Still's disease, inositis)

Table II. Risk of thrombotic events according the presence of positive antibodies (αCL and anti-β2GP1) in patients with capillaroscopic microhaemorrhages.

Predictor	Odds ratio	95% Confidence intervals	p-value
Positive αCL	10.909	3.591-33.144	<0.001
Positive β2GP1	9.882	3.529-26.677	<0.001
Positive β2GP1-IgG	16.296	5.364-49.511	<0.001
Positive β2GP1-IgM	5.515	1.135-26.783	0.076
Positive β2GP1-IgA	2.278	0.427-12.161	0.650

Table III. Association of the type of the thrombotic event with the presence of αCL and anti-β2GP1 Abs in patients with capillaroscopic microhaemorrhages and thrombotic events (total number 20 patients).

Autoantibodies	no. of pts with arterial thrombotic event(s)	no. of pts with venous thrombotic event(s)	χ^2	p-value
αCL positive	5	4	0.109	0.642
anti-β2-GP1 positive	9	3	0.449	0.356

clinical history of thrombosis exhibited a capillaroscopic pattern suggestive of antiphospholipid syndrome while repeatedly seronegative for antiphospholipid autoantibodies.

Discussion

In patients with capillaroscopic microhaemorrhages, the presence of αCL and anti-β2 GP1 Abs (IgM and IgG) seem to independently correlate significantly with clinical thromboses, in accordance with studies reporting similar results in patients with clinical lupus (7-9). The observation that, although IgA-β2GP1 Abs were detected in patients with microangiopathy (*i.e.* capillaroscopic microhaemorrhages), they lacked any significant association with thrombotic complications, suggests, that additional factors besides autoimmunity may be needed for the development of clinical thrombotic events.

The 17.69% (after the exclusion of patients with primary APS) of newly diagnosed patients with secondary laboratory APS (positive antiphospholipid antibodies as an incidental finding) indicates that capillaroscopy may act synergistically to contribute to the diagnosis of APS. The importace of a supportive role of capillaroscopy in the diagnosis of APS is fortified by recent observations of seronegative (in terms of currently identified and characterised antiphospholipid antibodies) patients with clinical manifestations of APS (10,

11). Seronegative APS may translate in the presence of autoantigens and autoantibodies not characterised to-date. In this case, capillaroscopy could provide a clinical research tool for further identification of analogous molecules.

The presence of αCL antibodies has been previously associated with abnormal capillaroscopic findings (4). To our knowledge, no specific capillaroscopic patterns have been described in patients with anti-beta 2 glycoprotein 1 antibodies. Setting out from these preliminary observations, studies involving preselected subpopulations of patients will help define the potential associations of specific autoantibodies with the type and/ or severity of capillaroscopic abnormalities (12).

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