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Thromboses in tuberculosis are linked to antiphosphatidylethanolamine antibodies levels: A cross-sectional study

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Abstract:

Venous thromboses have been associated with tuberculosis, but the relationship with circulating anticoagulant has not been studied yet. In a cohort of 48 patients with tuberculosis, 22.9 % of them presented with venous thromboses significantly associated with dose dependent level of antiphosphophatidyl-ethanolamine antibodies.

Introduction:

Tuberculosis remains a frequent and serious worldwide disease that continues to affect public health. Among the complications that have long been largely neglected is venous thrombosis (VTE), which includes pulmonary embolism and deep or superficial venous thrombosis [1,2]. Several case reports and small series have reported significant associations between VTE and tuberculosis and have identified tuberculosis as a risk factor for thrombosis [1]. In Dentan et al., a 2.07 % prevalence of VTE in tuberculosis was reported and the authors estimated that the risk of thrombosis in tuberculosis was equivalent to neoplasia [1].

The mechanism by which VTEs occur in tuberculosis is still poorly understood. Usually, the infectious process itself is considered a risk of VTE [3,4], and most authors suggest that the origin is based on Virchow's Triade, defined as an endothelial lesion associated with extrinsic compression and a pro-inflammatory state stimulating the blood-craze pathways, to produce a hypercoagulable state [3,4]. In Q fever, *Coxiella burnetii* infection, deep vein thrombosis is mediated by anti-cardiolipid (aCL) IgG [5]. In tuberculosis, a significant elevation of antiphospholipid (aPL) antibodies such as, aCl IgM and anti beta2-glycoprotein 1 (aB2GP1) IgM and IgG was reported, but the link with thrombosis was not established [6]. To the best of our knowledge, there is no evidence in the literature of the association between elevation of aPL and VTE during tuberculosis.

The aim of this study was to investigate a putative link between aPL and VTE in patients with tuberculosis in order to better assess the risk of VTE.

Patient and methods:

We performed a cross-sectional study assessing the association of aPL and the occurrence of VTE according to the STROBE statements. The study was conducted between January 2017 and May 2018 in the Institute for infectious disease (Méditerranée Infection) at the Assistance Publique-Hôpitaux de Marseille, France. We retrospectively collected data issued from medical records of patients suffering from active tuberculosis. The diagnosis of tuberculosis was confirmed when a bacterial culture with MALDI-TOF identification or *Mycobacterium tuberculosis* PCR was positive in the samples (sputum, bronchial aspiration, stool, biopsy). The diagnosis of VTE was confirmed when, during the length of stay, a doppler ultrasound and / or computed tomography revealed a thrombus.

For each patient, we recorded sex, age, length of stay, co-morbidities, country of birth, phototype, OMS score (performance status), presence of antithrombotic prophylaxis, presence of VTE, type of VTE, location of tuberculosis, platelets count, C-reactive-protein levels, complement assay, lupus anticoagulant (LA), IgG / IgM isotypes of aCL, aB2GP1 and aPE .

For all patients in our tuberculosis cohort, blood samples were collected at the time of diagnosis. The sera were kept frozen at -80 ° C until further analysis for aPL detection.

aCL antibody ELISA: IgG and IgM aCL antibodies were detected with an in-house previously described ELISA. The results were expressed in anti-IgG phospholipid units/ml (GPLU/ml) and anti-IgM phospholipid units/ml (MPLU/ml) for IgG and IgM aCL, respectively. The cut-off values were 22 GPLU/ml and 10 MPLU/ml for IgG aCL and IgM aCL, respectively [7].

aβ2GP1 antibody ELISA: IgG and IgM anti-β2GP1 antibodies were detected by using a commercially available ELISA (Orgentec Diagnostika GmbH, Mainz, Germany). Cut-off for positivity for both IgG and IgM aB2GP1 antibodies was 8 U/ml according to manufacturer's instructions.

aPE ELISA: IgG and IgM aCL antibodies were detected with an in-house previously described ELISA. The cut-off levels for IgG-aPE and IgM-aPE were 18 and 59 U/mL respectively[8].

Statistical analyses

To study the association between each aPL and VTE, categorical variables were compared using mid-p test and quantitative variables were compared using Mann-Whitney test. Multivariate comparative analyzes were performed to determine the independent predictors associated with VTE among variables with a $p < 0.20$ and/or relevant for thrombosis. A dose-dependent relationship between each aPL levels and VTE was assessed using Receiving Operating Curve (ROC) analysis. Positive and negative predictive values were examined to determine clinically relevant thresholds to support clinical decision-making. All tests were two-sided and a p-value $< .05$ was considered significant. Statistical analysis were performed using SPSS 20 software (IBM, Paris, France) and XLSTAT v2018.5 (Addinsoft, Paris, France).

This work is carried out as part of a research cohort validated by the local ethics committee: N°ID-RCB 2012-A01598-35.

Results:

Characteristic of populations:

We included 48 patients with active tuberculosis. According our criteria, 37 cases without VTE and 11 cases with VTE were identified**.** Among the latter, we found 9 cases of pulmonary embolism, 4 deep venous thrombosis and 2 patients presented multiple thrombosis. No deaths were recorded in either group. Patients in the VTE group were treated with curative anticoagulation for 3 months without any particular complications. The overall prevalence of VTE in our cohort was 22.9 %. No significant differences were observed for the following variable between the 2 groups: sex, age, co-morbidities, phototype, country of birth, C-Reactive Protein and platelets counts. An OMS score (Performance status) greater than 2 *(p = 0.004)* and an excess extra-pulmonary tuberculosis *(p = 0.01)* was recorded in the VTE group.

aPL and thrombosis:

aPE levels were higher in the VTE group (median [IQR], 22.27 [15.33-38.64] vs 11.64 [8.01-20.92], two-sided Mann-whitney test p = 0.012). The ROC curve analysis of aPE association with VTE occurrence revealed an area under curve (AUC) of 0.81 (95%CI 0.63-0.98) with p = 0.001. In addition, we identified a threshold of 12.78 U/ml below which the negative predictive value is 100% (no VTE occurred below this threshold). Above this threshold, the positive predictive value increased almost linearly, and for a threshold of 18 U/ml (normal threshold for our laboratory), the positive predictive value was 50% and the negative predictive value was 87%. Only two patients had more than 75 U/ml and both presented a thrombosis (positive predictive value of 100%). Surprisingly, these two patients were the most severe in the series with both a pulmonary embolism and multiple thromboses.

Univariate analysis showed that the presence of LA (p = 0.02) and positive aPE IgG (p=0.0043) were associated with VTE. There were no statistical differences between two groups with aCL (IgM, IgG), aB2GP1 (IgM, IgG). In a logistic regression model including VTE as the outcome and age, gender, ethnic group, co-morbidities, OMS score (performans status), antithrombotic prophylaxis as potential predictors and tuberculosis location, only aPE IgG (2.6; 1.15-174.39, p = .038) were independent predictors of thrombosis **(Fig1).**

Discussion:

We observed a significant dose-dependent association between aPE IgG and venous thrombosis during tuberculosis. The originality of this work lies in the demonstration of a possible link between the occurrence of VTE and aPE IgG levels in tuberculosis patient, suggesting new hypothesis about the mechanism of VTE in this situation. This association is consistent with the literature, and aPE IgG has been associated with thrombosis in other contexts. First, aPE have been clearly identified as another prothrombotic factor in primary antiphospholipid syndrome or systemic lupus erythematosus [9]. It has proven to be interesting because it readjusts the diagnosis, especially when antiphospholipid syndrome is not sufficiently documented by conventional aPL abnormalities[9]. Phosphatidyl-ethanolamine is present on the luminal endothelial surface, and functions as a critical anticoagulant, suggesting that the prothrombotic activity of aPE is consistent with VTE [10].

In addition, aPE have recently been shown to be significantly elevated in patient with tuberculosis[6] as in mouse model[11]. To the best of our knowledge, no infections other than tuberculosis have been associated with aPE to date. In the study of Sartain et al [12], phosphatidyl-ethanolamine structure changes according to the multiplication phase of *M. tuberculosis*. In the logarithmic phase, the unsaturated form with 34 carbons is the most abundant. Our detection assay uses phosphatidyl-ethanolamine of yolk egg containing unsaturated 34 carbon phosphatidyl-ethanolamine (C34:0). The fact that *M.tuberculosis* is rich in lipids and in particular in phosphatidyl-ethanolamine structurally identical to that used in the test could be an argument for a specific immunization against *M. tuberculosis*.

Thrombosis in tuberculosis is a frequent complication that exposes patients to an increased risk of death, longer hospital stays and a significant risk of drug interactions, especially with rifampicin. Our findings are preliminary and need to be confirmed by a larger

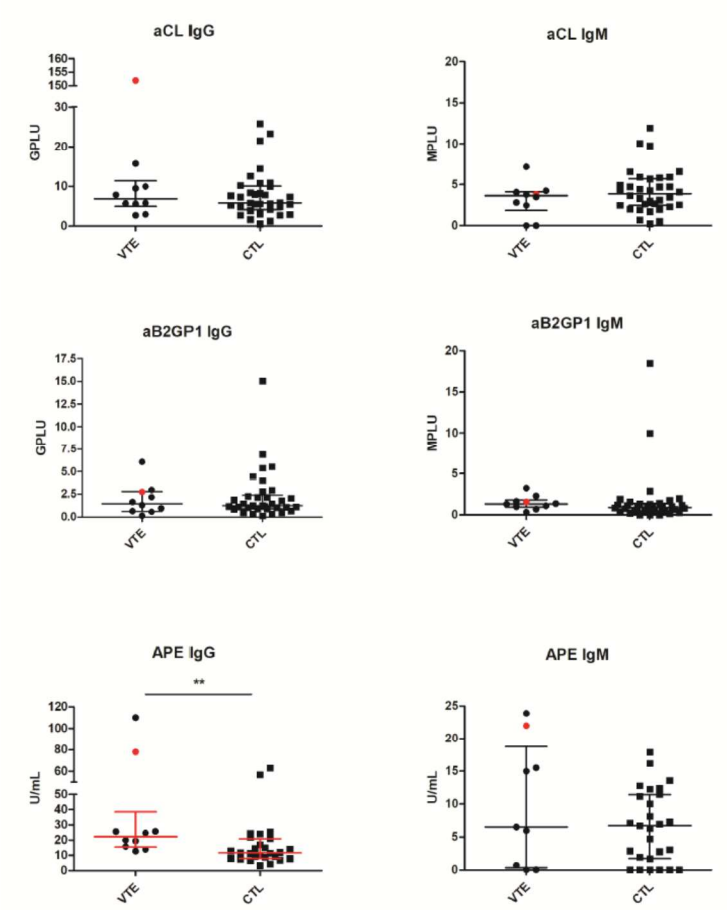
prospective cohort. However, our results suggest that patients with tuberculosis and aPE IgG > 18U/ml should be placed on preventive anticoagulation therapy.

Declaration of conflict of interest: The authors have no conflict of interest to declare

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Annexes:

Fig 1 : antiphospholipid antibodies and thrombosis during tuberculois



	VTE N=11 (%)	Absence of VTE N=37 (%)	Total N= 48 (%)	Univariate p-value	Multivariate p-value
Positive IgG aCL[c] (> 15 IU/ml)	2/10 (20%)	3/34 (9%)	5/11 (11.4%)	.19	NS
Positive IgM aCL[c] (> 15 IU/ml)	0	0	0	NA	NS
Positive IgG aB2GPI[c] (> 8 IU/ml)	0	1 /34 (2.94%)	1/45 (2.22%)	0.29	NS
Positive IgM aB2GPI[c] (> 8 IU/ml)	0	2/34 (5.88%)	2/45 (4.44%)	0.38	NS
Positive IgG aPE[d] (> 18 IU/ml)	7/32 (21.87%)	7/10 (70%)	14/43 (32.55%)	0.043	.038
Positive IgM aPE[d] (> 55 IU/ml)	0	0	0	NA	NS
Presence of a lupus anticoagulant[e]	4/8 (50%)	1/18 (5.55)	5/26 (19.23%)	0.02	NS

NS: not significant, [a]two-sided mid-p test (comparison between VTE and absence of VTE), [b] Logistic regression model including the antiphospholipid marker and age, sex, performance status, ethnic group, comorbities [c] Data was missing for 1 individual with VTE and 3 in those without VTE, [d] Data was missing for 1 individual with VTE and 5 in those without VTE, [e] Data was missing for 3 individual with VTE and 19 in those without VTE.

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