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

3

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20 **Abstract:**

21 Venous thromboses have been associated with tuberculosis, but the relationship with
22 circulating anticoagulant has not been studied yet. In a cohort of 48 patients with tuberculosis,
23 22.9 % of them presented with venous thromboses significantly associated with dose
24 dependent level of antiphosphatidyl-ethanolamine antibodies.

25 **Introduction:**

26 Tuberculosis remains a frequent and serious worldwide disease that continues to affect
27 public health. Among the complications that have long been largely neglected is venous
28 thrombosis (VTE), which includes pulmonary embolism and deep or superficial venous
29 thrombosis^{1,2}. Several case reports and small series have reported significant associations
30 between VTE and tuberculosis and have identified tuberculosis as a risk factor for thrombosis
31¹. In Dentan et al., a 2.07 % prevalence of VTE in tuberculosis was reported and the authors
32 estimated that the risk of thrombosis in tuberculosis was equivalent to neoplasia¹.

33 The mechanism by which VTEs occur in tuberculosis is still poorly understood.
34 Usually, the infectious process itself is considered a risk of VTE^{3,4}, and most authors suggest
35 that the origin is based on Virchow's Triade, defined as an endothelial lesion associated with
36 extrinsic compression and a pro-inflammatory state stimulating the blood-clot pathways, to
37 produce a hypercoagulable state^{3,4}. In Q fever, *Coxiella burnetii* infection, deep vein
38 thrombosis is mediated by anti-cardiolipid (aCL) IgG⁵. In tuberculosis, a significant elevation
39 of antiphospholipid (aPL) antibodies such as, aCl IgM and anti beta2-glycoprotein 1
40 (aB2GP1) IgM and IgG was reported, but the link with thrombosis was not established⁶. To
41 the best of our knowledge, there is no evidence in the literature of the association between
42 elevation of aPL and VTE during tuberculosis.

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

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48 **Patient and methods:**

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

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

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

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

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

72 aPE ELISA: IgG and IgM aCL antibodies were detected with an in-house previously
73 described ELISA. The cut-off levels for IgG-aPE and IgM-aPE were 18 and 59 U/mL
74 respectively⁸.

75

76 **Statistical analyses**

77 To study the association between each aPL and VTE, categorical variables were compared
78 using mid-p test and quantitative variables were compared using Mann-Whitney test.

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

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

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95 **Results:**

96 Characteristic of populations:

97 We included 48 patients with active tuberculosis. According our criteria, 37 cases
98 without VTE and 11 cases with VTE were identified. Among the latter, we found 9 cases of
99 pulmonary embolism, 4 deep venous thrombosis and 2 patients presented multiple
100 thrombosis. No deaths were recorded in either group. Patients in the VTE group were treated
101 with curative anticoagulation for 3 months without any particular complications. The overall
102 prevalence of VTE in our cohort was 22.9 %. No significant differences were observed for the
103 following variable between the 2 groups: sex, age, co-morbidities, phototype, country of birth,
104 C-Reactive Protein and platelets counts. An OMS score (Performance status) greater than 2 (p
105 = 0.004) and an excess extra-pulmonary tuberculosis ($p = 0.01$) was recorded in the VTE
106 group.

107 aPL and thrombosis:

108 aPE levels were higher in the VTE group (median [IQR], 22.27 [15.33-38.64] vs 11.64
109 [8.01-20.92], two-sided Mann-whitney test $p = 0.012$). The ROC curve analysis of aPE
110 association with VTE occurrence revealed an area under curve (AUC) of 0.81 (95%CI 0.63-
111 0.98) with $p = 0.001$. In addition, we identified a threshold of 12.78 U/ml below which the
112 negative predictive value is 100% (no VTE occurred below this threshold). Above this
113 threshold, the positive predictive value increased almost linearly, and for a threshold of 18
114 U/ml (normal threshold for our laboratory), the positive predictive value was 50% and the
115 negative predictive value was 87%. Only two patients had more than 75 U/ml and both
116 presented a thrombosis (positive predictive value of 100%). Surprisingly, these two patients
117 were the most severe in the series with both a pulmonary embolism and multiple thromboses.

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

124 **Discussion:**

125 We observed a significant dose-dependent association between aPE IgG and venous
126 thrombosis during tuberculosis. The originality of this work lies in the demonstration of a
127 possible link between the occurrence of VTE and aPE IgG levels in tuberculosis patient,
128 suggesting new hypothesis about the mechanism of VTE in this situation. This association is
129 consistent with the literature, and aPE IgG has been associated with thrombosis in other
130 contexts. First, aPE have been clearly identified as another prothrombotic factor in primary
131 antiphospholipid syndrome or systemic lupus erythematosus⁹. It has proven to be interesting
132 because it readjusts the diagnosis, especially when antiphospholipid syndrome is not
133 sufficiently documented by conventional aPL abnormalities⁹. Phosphatidyl-ethanolamine is
134 present on the luminal endothelial surface, and functions as a critical anticoagulant,
135 suggesting that the prothrombotic activity of aPE is consistent with VTE¹⁰.

136 In addition, aPE have recently been shown to be significantly elevated in patient with
137 tuberculosis⁶ as in mouse model¹¹. To the best of our knowledge, no infections other than
138 tuberculosis have been associated with aPE to date. In the study of Sartain et al¹²,
139 phosphatidyl-ethanolamine structure changes according to the multiplication phase of *M.*
140 *tuberculosis*. In the logarithmic phase, the unsaturated form with 34 carbons is the most
141 abundant. Our detection assay uses phosphatidyl-ethanolamine of yolk egg containing
142 unsaturated 34 carbon phosphatidyl-ethanolamine (C34:0). The fact that *M.tuberculosis* is
143 rich in lipids and in particular in phosphatidyl-ethanolamine structurally identical to that used
144 in the test could be an argument for a specific immunization against *M. tuberculosis*.

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

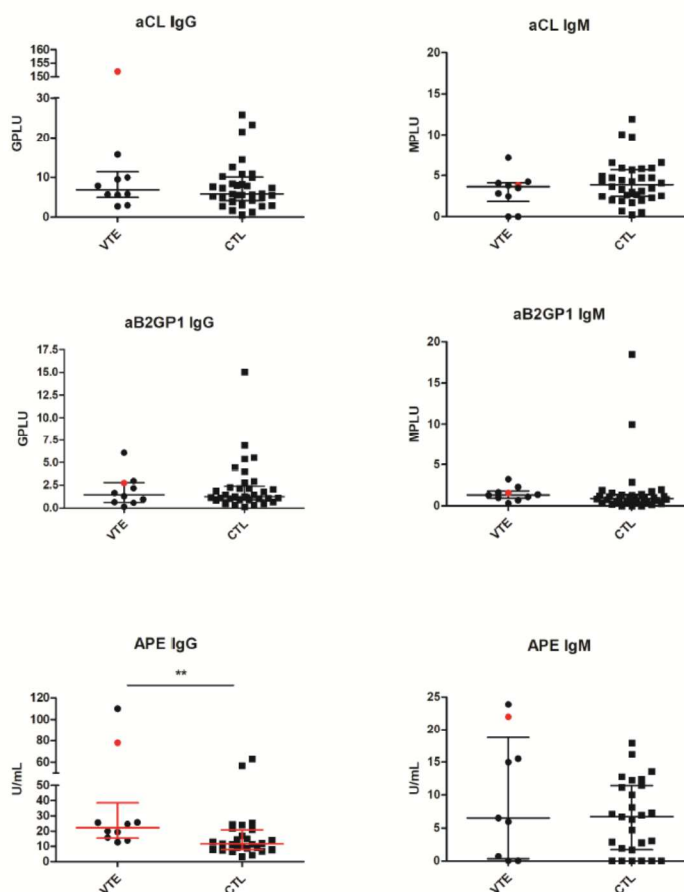
148 prospective cohort. However, our results suggest that patients with tuberculosis and aPE IgG
149 > 18U/ml should be placed on preventive anticoagulation therapy.

150

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

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Fig 1 : antiphospholipid antibodies and thrombosis during tuberculosis



	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, ^atwo-sided mid-p test (comparison between VTE and absence of VTE), ^bLogistic regression model including the antiphospholipid marker and age, sex, performance status, ethnic group, comorbidities ^cData was missing for 1 individual with VTE and 3 in those without VTE, ^dData was missing for 1 individual with VTE and 5 in those without VTE, ^eData was missing for 3 individual with VTE and 19 in those without VTE.

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