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Vascular endothelial growth factor, tissue factor, coagulation and fibrinolysis markers in slow-flow vascular malformations: a prospective study of treatment with sirolimus

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1 **VEGF, tissue factor, coagulation and fibrinolysis markers in slow-flow**
2 **vascular malformations: a prospective study of sirolimus intervention**
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1 Dear Editor,

2 Slow-flow vascular malformations (VMs), particularly venous VMs, might get
3 complicated by intra-malformative thrombosis linked to localized intravascular coagulopathy
4 (LIC).¹⁻³ Several studies have shown the effectiveness of sirolimus on slow-flow VMs but its
5 effect on coagulation has been poorly studied.³ Moreover, vascular endothelial growth factor
6 A and C (VEGF-A and C) are known for their pro-angiogenic/lymphangiogenic activities
7 respectively and sirolimus may reduce their levels.⁴ In this study, we investigated blood levels
8 of VEGF-A and C, tissue factor (TF) and coagulation markers in children with slow-flow
9 VMs before and after sirolimus.

10 Blood samples were collected from 59 children aged 6 to 18 years, included in the
11 multicenter phase II trial PERFORMUS, that assessed the efficacy and safety of sirolimus in
12 children with slow-flow VMs.⁵ Children first underwent an observational period, then, at a
13 randomized time between 4 and 8 months after inclusion, received sirolimus until month 12
14 (M12).⁵

15 Blood samples were collected at least twice: at month 0 (M0; baseline) and M12. LIC
16 biomarkers (D-dimer, fibrinogen, factor V [FV], platelets) and plasma levels of VEGF-A,
17 VEGF-C and TF were measured.

18 We investigated: levels of biomarkers at baseline; changes in biomarker levels with
19 sirolimus treatment (between M12 and M0); and biomarker levels according to response to
20 sirolimus. We divided patients into 2 categories according to global response to treatment
21 (assessed by the physician on a 0 to 10 visual analog scale [VAS]): VAS > 7/10 on at least one
22 visit was “high response”; otherwise, the response was “low/medium”. Global response
23 assessed pain, functional/aesthetic impairments, perception of changes in the VM volume,
24 oozing/bleeding.

25 Among the 59 children included (mean age 11.6 years, 35 [59.3%] females), 22 had
26 venous VMs, 18 lymphatic VMs and 19 combined veno-lymphatic VMs. At M12, 26 (44.1%)
27 participants showed high response to sirolimus and 33 (55.9%) low/medium response.
28 Patients with lymphatic VMs were better responders than those with venous and combined
29 VMs (rate of high responders respectively 61.1%, 31.8% and 42.1%). Five patients with
30 venous (n=4) or combined (n=1) VMs experienced painful superficial venous thrombosis (3
31 during sirolimus treatment).

32 At baseline, D-dimer level was increased in venous and combined VMs (median 1 159
33 and 993 ng/mL, respectively; normal < 500 ng/mL), as compared with lymphatic VMs
34 (median 500 ng/mL, p=0.021). Fibrinogen and FV levels were lower in venous and combined

1 VMs (although within the normal range). Overall, 81.8% of patients with venous VMs (n=18)
2 and 63.1% with combined VMs (n=12) had increased D-dimer; 2 had low fibrinogen. There
3 were no significant differences for the other biomarkers between the subgroups of VMs.

4 During sirolimus treatment (**Table 1**), the venous VM group experienced a significant
5 increase in FV and fibrinogen levels (although median levels remained in the normal range)
6 and a decrease in D-dimer level (median from 1 159 to 553 ng/mL), although not statistically
7 significant. Also, levels of VEGF-A increased at M12 in venous VMs (from 14.5 to
8 23 pg/mL). No significant changes were observed in other VMs subgroups. Baseline
9 biomarker levels in the subgroups of VMs were not associated with response to treatment.

10 Our study shows that in children, slow-flow VMs with a venous component are
11 associated with coagulation abnormalities which are improved by sirolimus. Indeed, TF,
12 which triggers the coagulation cascade, is inhibited by sirolimus, thus decreasing D-dimer
13 level.⁶ These abnormalities are not observed in lymphatic VMs and slightly in combined
14 VMs, probably because the lymphatic component is often predominant in children. As venous
15 VMs are constituted of large ectasic vessels, increase in D-dimer is a pathophysiological
16 consequence to chronic activation of coagulation. Dompmartin et al. found that elevated D-
17 dimer level was associated with venous VMs (sensitivity 42.6%, specificity 96.5%) in 280
18 patients (mean age 26.8 years).¹ Median level of D-dimer was similar to that in our study,
19 although we included only children.

20 Data on coagulation markers in children with VMs are scarce. Two studies involving
21 24 and 62 children with venous VMs reported elevated D-dimer in 33% and 39% of cases
22 respectively.^{2,7} The proportion of patients with increased D-dimer in venous VMs was higher
23 in our study (81.8%).

24 VEGF-A level significantly increased in the venous VMs after sirolimus. Trujillo-
25 Vargas et al., observed similar results in evaluating eyedrops of rapamycin in mice, with an
26 upregulation of VEGF-A level in their tears.⁸ Biological significance of this result remains
27 however unknown. Regarding VEGF-C level, we did not find any significant changes in any
28 subgroups of VMs. Overall we did not clearly evidence predictive biomarkers of response to
29 sirolimus. However, the study was limited by the small number of samples analyzed.

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2 National Program of Clinical Research [PHRC-N], 2014). No industrial support was involved.

3 **Conflicts of interest:** None to declare.

4 **Data availability:**

5 Deidentified individual participant data will be made available, in addition to study protocols,
6 the statistical analysis plan, and the informed consent form upon publication to researchers
7 who provide a methodologically sound proposal for use in achieving the goals of the
8 approved proposal. A written agreement must be signed with the University Hospital of Tours
9 and the coordinator of the study. Proposals should be submitted to dpo@chu-tours.fr

10 **Ethical approvals:** The protocol was approved by the institutional review board of the
11 University Hospital Centre of Tours (#2015-R18) and received authorization from ANSM
12 (Agence Nationale de Sécurité du Médicament et des produits de santé).

13 **Trial registration:** ClinicalTrials.gov Identifier: NCT02509468, first received: July 15, 2015

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1 **Table 1. Biomarker levels at baseline and the end of sirolimus treatment (month 12) by type of VM**

	Overall*			Venous VMs		
	M0	M12	P-value	M0	M12	P-value
Platelet count, G/L (normal: 150–400)	259 [210;312]	272.5 [219;322.8]	0.112	259 [223;333]	289 [228;335]	0.156
Fibrinogen, g/L (normal: 1.6–4)	2.7 [2.3;3.2]	3.2 [2.8;3.7]	< 0.001	2.3 [2;2.6]	2.8 [2.5;3.3]	0.001
D-dimer, ng/mL (normal <500)	670 [495;2 180]	525 [404;1 226]	0.098	1159 [663;3 883]	553 [414;3 043]	0.379
Factor V, % (normal: 60–120)	96 [88;107]	110 [101;124]	< 0.001	95 [85;104]	107 [90;121]	0.027
VEGF-A, pg/mL**	15 [10;24]	19 [11;31]	0.130	14.5 [10;22.2]	23 [15;29]	0.038
VEGF-C, pg/mL**	823.9 [508.9;1 051]	777.8 [484.7;1 001.6]	0.952	823.9 [454.6;947.7]	863.5 [373.9;1 250]	0.252
Tissue factor, pg/mL**	47 [42.2;54.5]	47 [42;51]	0.061	48 [44;58]	47 [42;51]	0.078
	Lymphatic VMs			Veno-lymphatic VMs		
	M0	M12	P-value	M0	M12	P-value
Platelet count, G/L (normal: 150–400)	257 [202;293]	236 [214;291]	0.518	266 [209;311]	275 [202;306]	0.737
Fibrinogen, g/L (normal: 1.6–4)	2.8 [2.5;3.4]	3.2 [2.8;3.5]	0.112	3.1 [2.7;3.6]	3.7 [3.2;4]	0.258
D-dimer, ng/mL (normal <500)	500 [443;548]	500 [500;663]	0.894	993 [490;1 810]	810 [245;1 503]	0.135
Factor V, % (normal: 60–120)	111 [103;120]	117 [108;124]	0.068	92 [86;97]	107 [101;127]	0.036
VEGF-A, pg/mL**	15 [12;16.2]	15.5 [11.8;22.5]	0.350	22 [9.2;26.5]	22 [9;33]	0.362
VEGF-C, pg/mL**	763[543.2;965.6]	722.9 [646.8;884.7]	0.67	877.3 [703.7;1 130.7]	813.7 [547;987]	0.432
Tissue factor, pg/mL**	47 [43.2;55]	49.5 [46.3;52.5]	0.706	44 [40.5;48]	42 [40.2;44.5]	0.023

2 *Data are median [Q1;Q3]; VEGF: vascular endothelial growth factor ;*3 **Platelet count, fibrinogen, D-dimer and factor V were analyzed for the 59 patients included. VEGF-A, VEGF-C and Tissue factor were available for 55*4 *patients (17 with lymphatic VMs, 20 with venous VMs and 18 with veno-lymphatic VMs). **Normal values are unknown*