# Increased soluble thrombomodulin influences plasma clot formation in patients with mild to moderate bleeding tendency

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## Background

A majority of patients with a mild bleeding disorder (MBD) remains without a diagnosis, despite thorough hemostatic investigations (bleeding of unknown cause, BUC).[1] Recently, a coagulopathy with posttraumatic bleeding caused by 100-fold enhanced levels of soluble thrombomodulin (TM) was reported in several cases.[2,3]

## Objectives

We investigated TM levels in a large and well-characterized cohort of patients with MBDs from the Vienna Bleeding Biobank and healthy controls, and how high TM levels affect thrombin generation and plasma clot formation.

## Patients and Methods

TM and thrombin generation (TG) were measured using commercially available kits (ab46508 – TM (CD141) Human ELISA Kit – Abcam UK; TG Technothrombin, Technoclone, Austria). Plasma clot formation/lysis was assessed according to SCC recommendations of the ISTH.[4]

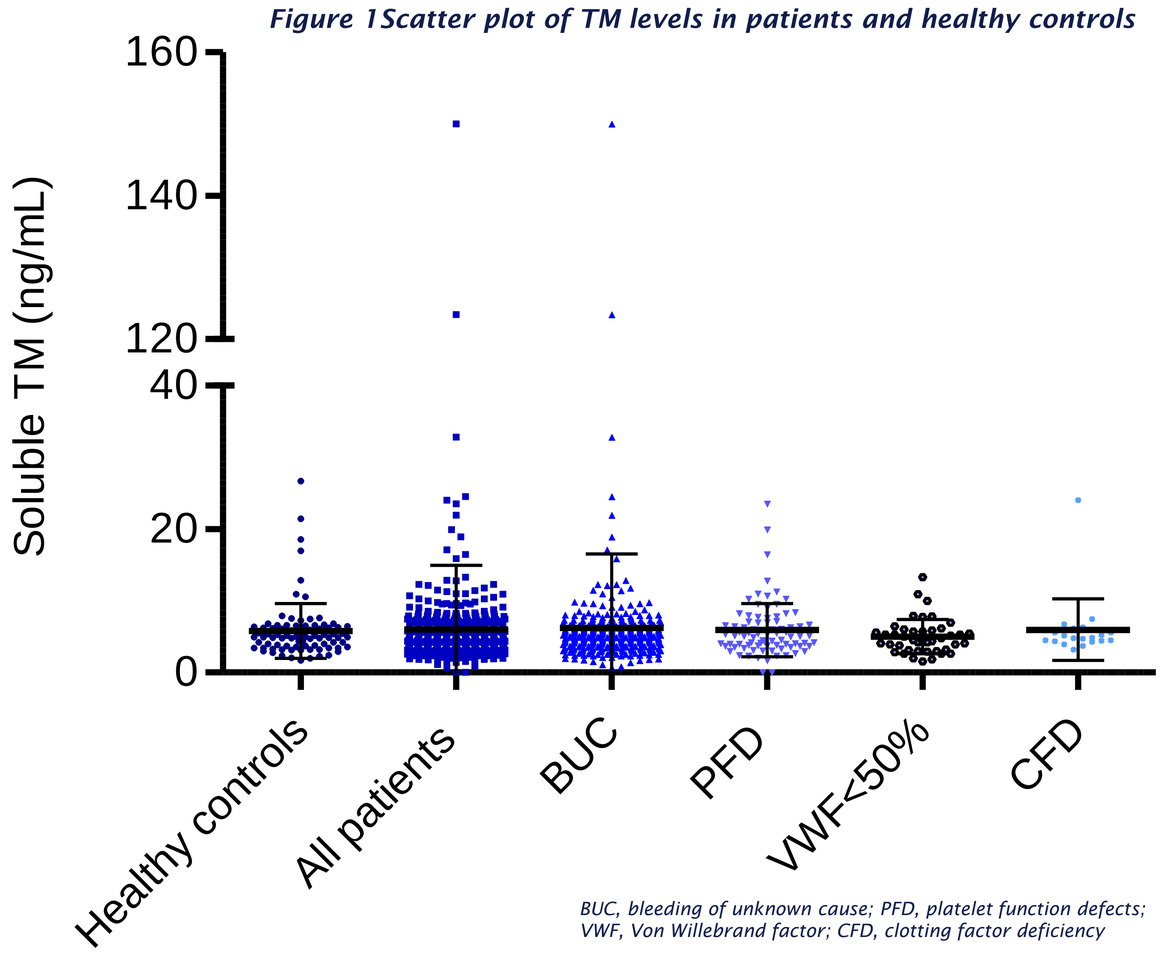
***Table 1 Characteristics of patients and healthy controls***

| | All patients (n=507) | Controls (n=90) |
|---|---|---|
| **Female, n (%)** | 412 (81.3) | 73 (81.1) |
| **Blood group O, n (%)** | 254 (50.1) | 21 (23.3) |
| **Positive family history, n (%)** | 184 (36.3) | na |
| | *mean (SD)* | *mean (SD)* |
| **Age, years** | 41.6 (15.8) | 42.6 (15.2) |
| **BMI, kg/m²** | 24.2 (4.5) | 23.9 (7.1) |
| **Hemoglobin, g/dL** | 13.6 (1.3) | 14.4 (4.2) |
| **Platelet count, x10⁹/L** | 250.9 (66.6) | 260.9 (48.6) |
| **Fibrinogen, mg/dL** | 316.6 (72.6) | 292.8 (63.1) |
| | *median (IQR)* | *median (IQR)* |
| **aPTT-STA, seconds** | 35.8 (33.6-38.7) | 34.7 (32.9-36.1) |
| **Prothrombin time, %** | 95.0 (88.0-102.0) | 101.0 (91.5-109.0) |
| | *mean (SD)* | *mean (SD)* |
| **Vicenza bleeding score** | 5.7 (3.0) | 0 (0) |
| **ISTH BAT** | 6.5 (3.5) | na |

## Results

**TM was not altered in 507 patients**, including 358 BUC patients, with MBD when comparing to 90 sex- and age-matched healthy controls (median [IQR] 5.0 [3.8-6.3] vs. 5.1 [3.7-6.4] ng/mL, multiple regression with adjustment for sex p=0.801; Figure 1). **Also, in the BUC patients no difference was found** (TM: 5.05 [3.8-6.3] ng/mL, multiple regression after adjustment for sex p= 0.695). To identify outliers of TM levels in our patients, a cut-off according to the 95$^{th}$ percentile of TM in healthy controls (≥ 14.7 ng/mL) was defined. No increased number of patients above the predefined cut-off was identified (OR [95% CI]: 1.9 [0.6-6.1]). Nevertheless, **2 BUC patients had clearly elevated TM levels (150.1 and 123.4 ng/mL, reference range: 2.9-7.6)**, but TG and plasma clot properties were not significantly altered.

***Figure 1Scatter plot of TM levels in patients and healthy controls***




*BUC, bleeding of unknown cause; PFD, platelet function defects; VWF, Von Willebrand factor; CFD, clotting factor deficiency*

We identified a **prolonged time to peak (TTP) in plasma clot analysis** between patients with TM levels above vs. below the cut-off (mean (SD): 28.7 (31.7) vs. 20.0 (7.4) minutes, p<0.001), which was also significant in subgroup analysis of patients with BUC (Table 2). In the **thrombin generation assay no differences could be found**. Levels of **TM did not correlate with neither the Vicenza ($r_s$=-0.052, p=0.239) nor the ISTH bleeding score ($r_s$=-0.057, p=0.368)**.

***Table 2 Plasma clot properties according to soluble TM levels***

| | TM <95$^{th}$ percentile (< 14.7 ng/mL) | TM ≥ 95$^{th}$ percentile (≥ 14.7 ng/mL) | P | BHC |
|---|---|---|---|---|
| | *mean (SD)* | *median (SD)* | | |
| ***All patients*** | *n= 495* | *n= 12* | | |
| **Lag time, min, median [IQR]** | 10.7 (7.7-24.3) | 9.7 (6.6-18.2) | .898 | ns |
| **ΔAbs, OD 405nm, mean [SD]** | 0.72 (0.18) | 0.64 (0.15) | .114 | ns |
| **TTP, min, mean [SD]** | 20.0 (7.4) | 28.7 (31.7) | <.001 | <.05 |
| **Vmax, OD/min, mean [SD]** | 0.13 (0.05) | 0.11 (0.05) | .175 | ns |
| **CLT, min, median [IQR]** | 15.6 (13.1-18.9) | 13.9 (12.6-19.9) | .798 | ns |
| ***BUC*** | *n=350* | *n=8* | | |
| **Lag time, min, median [IQR]** | 10.3 (7.2-13.7) | 10.9 (7.5-18.2) | .397 | ns |
| **ΔAbs, OD 405nm, mean [SD]** | 0.73 (0.17) | 0.61 (0.15) | .046 | ns |
| **TTP, min, mean [SD]** | 19.4 (6.9) | 32.2 (37.1) | <.001 | <.05 |
| **Vmax, OD/min, mean [SD]** | 0.14 (0.05) | 0.10 (0.04) | .039 | ns |
| **CLT, min, median [IQR]** | 15.7 (13.3-19.3) | 16.5 (13.3-19.9) | .871 | ns |

*SD, standard deviation; IQR, interquartile range; ΔAbs, maximum absorbance at plateau; Vmax, the maximum rate of turbidity increase; TTP, time to peak; AUC, area under the curve; BHC, Bonferroni-Holm correction*

## Conclusion

**Soluble TM was not increased in patients with MBD or patients with BUC in comparison to healthy controls. High levels of TM lead to a prolonged TTP in the plasma clot formation assay.** Two patients with clearly elevated TM levels were identified, though levels were not as high as in reported cases of TM-associated coagulopathy. Thus, **TM does not appear to have an impact on bleeding in patients with MBD and/or BUC.**

## References

[1]Mezzano et al. JTH. 2019; [2]Langdown et al. Blood. 2014; [3]Westbury. JTH. 2020; [4]Pieters et al. JTH. 2018

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