SUPPLEMENTARY APPENDIX

Generation of anti-idiotypic antibodies to detect anti-spacer antibody idiotopes in acute thrombotic thrombocytopenic purpura patients

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Supplemental methods

Animals

Animal experiments were approved by the Institutional animal Care and Use Committee of KU Leuven, Belgium (project number P055/2015). Mice were anesthetized using isoflurane/O_2 before subcutaneous or intra-peritoneal injections and retro-orbital venipuncture. Serum was obtained from blood samples by 1 hour incubation at 37°C and centrifugation at 13,400 rpm for 10 minutes. Serum samples were stored at -20°C.

Cloned human anti-spacer autoantibodies

Three cloned human anti-spacer autoantibodies with different epitopes and different inhibitory characteristics were used for the development of anti-idiotypic antibodies: anti-spacer autoantibody II-1[1], TTP73[2] and I-9[3]. Anti-spacer autoantibody II-1 targets the R568-F592-R660-Y661-Y665 epitope in ADAMTS13's spacer domain[4] and is a strong inhibitor of ADAMTS13 activity[1]. Anti-spacer autoantibody I-9 targets the R568-R660-Y661-Y665 epitope[4] and is a weak inhibitor of ADAMTS13 activity[1]. Anti-spacer autoantibody TTP73[2] recognizes an epitope in ADAMTS13 that does not overlap with the epitope of anti-spacer autoantibodies II-1 and I-9 (epitope at the amino acid level not known) and does not inhibit ADAMTS13 activity.

Immunization strategy and characterization of anti-II-1, anti-TTP73 and anti-I-9 antibodies

Immunization strategy

BALB/c mice (Janvier Labs, Le Genest-Saint-Isle, France) were immunized with cloned human anti-spacer autoantibodies II-1[1], TTP73[2], or I-9[3]. Briefly, for each antibody, mice were injected subcutaneously with 10 µg antibody (either II-1, TTP73, or I-9) in complete Freund's adjuvant (BD, Franklin Lakes, NJ, USA) at day 1 and intraperitoneally with 10 µg of antibody in incomplete Freund's adjuvant (BD) at day 14. Mice immunized with vehicle only were used as a negative control. Twenty-one days after the first immunization, blood samples were taken to detect the presence of murine anti-human II-1, TTP73 and I-9 antibodies in ELISA (see below). The immune response in mice was boosted at day 56 and 58 by injection of each antibody (either II-1, TTP73, or I-9). At day 59, the presence of murine anti-human II-1, TTP73 and I-9 antibodies in mouse sera was confirmed using ELISA (see below). At day 60, mice were sacrificed and their spleens were isolated. Spleen cells were fused with SP2/0 myeloma cells to generate hybridoma cells according to the method of Köhler and Milstein.[5] Fourteen days after fusion, media of the cultured hybridoma cells was screened for the presence of either anti-II-1, anti-TTP73 or anti-I-9 antibodies using ELISA (see below). Hybridoma cells positive for anti-II-1, anti-TTP73 or anti-I-9 antibodies were further cultured and anti-II-1, anti-TTP73 or anti-I-9 antibodies were purified from the culture medium using protein G sepharose affinity chromatography (ÄKTA, GE Healthcare, Waukesha, WI, USA). Antibody concentration was determined by measuring absorbance at 280 nm and antibody purity was checked via sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and protein blue staining (Westburg, Leusden, The Netherlands). The binding of the purified anti-II-1, anti-TTP73 or anti-I-9 antibodies to II-1, I-9 and TTP73 respectively was confirmed in ELISA (see below). ELISAs to identify the anti-idiotypic antibodies amongst the anti-II-1, anti-TTP73 or anti-I-9 antibodies are described below. The finally selected anti-idiotypic antibodies 17H9 (anti-II-1 antibody), 9G12 (anti-TTP73 antibody) and 7D10 (anti-I-9 antibody) were subcloned as described elsewhere[6] and purified as described above.

ELISA to study the binding of murine anti-II-1, anti-TTP73 and anti-I-9 antibodies to coated human anti-spacer autoantibodies II-1, TTP73 and I-9

A 96-well microtiter plate was coated with either anti-spacer autoantibody II-1 (1.5 µg/mL), TTP73 (5 µg/mL) or I-9 (1.5 µg/mL in carbonate/bicarbonate coating buffer; 50mM Na_2CO_3/$NaHCO_3$, pH 9.6) and incubated overnight at 4°C. Next, the plate was washed and blocked with 3% milk powder in phosphate buffered saline (PBS) (blocking buffer). Either sera (start dilution 10%, v/v) from mice injected with anti-

spacer autoantibody II-1, TTP73 or I-9 or media (start dilution, 17%, v/v) from the anti-II-1, anti-TTP73 and anti-I-9 producing hybridoma cells or purified murine anti-II-1, anti-TTP73 and anti-I-9 antibodies (start concentration 1 µg/mL) were added to the respectively II-1, TTP73 or I-9 coated 96-well microtiter plates and a 1 in 2 dilution series was made. For each plate, a serum sample (21 days post first immunization, 1/500 start dilution) of mice immunized with either II-1, TTP73 or I-9 respectively was used as a positive control. Sera samples taken before immunization, naïve culture media or anti-glycoprotein Ib antibody 6B4[7] were used as a negative control respectively. Bound anti-II-1, anti-TTP73, or anti-I-9 antibodies were detected using horse radish peroxidase (HRP)-labelled goat anti-mouse (GAM) antibodies (1/10,000; Jackson ImmunoResearch, West Grove, PA, USA). Colouring reaction was performed using ortho-phenylenediamine dihydrochloride (OPD) and H_2O_2 and stopped with 4M sulfuric acid. Absorbance was measured at 490 nm. Data for the II-1, TTP73 or I-9 ELISA's were expressed as relative absorbance values (mean ± SD, n=3) with absorbance of the respective positive controls (sera of mice immunized with either II-1, TTP73 or I-9) set as 1.

ELISA to study the binding of murine anti-II-1, anti-TTP73 and anti-I-9 antibodies to a pool of human IgG antibodies

Murine anti-II-1, anti-TTP73 and anti-I-9 antibodies targeting the conserved regions (Figure 1, grey) in human immunoglobulin G (IgG) antibodies were identified using ELISA. A pool of human IgG antibodies (5 µg/mL in carbonate/bicarbonate coating buffer; Sigma-Aldrich, Saint-Louis, MO, USA) was coated on a 96-well microtiter plate. Plates were blocked with blocking buffer and murine anti-II-1, anti-TTP73 and anti-I-9 antibodies were added (5 µg/mL) and a 1 in 2 dilution series was made. For each plate, a serum sample (21 days post first immunization, 1/500 start dilution) of mice immunized with either II-1, TTP73 or I-9 respectively was used as a positive control. The anti-glycoprotein Ib antibody 6B4[7] was used as a negative control. Bound murine anti-II-1, anti-TTP73 and anti-I-9 antibodies were detected with GAM-HRP (1/10,000; Jackson ImmunoResearch). Colouring reaction was performed as described above and absorbance was measured at 490 nm. Data for the II-1, TTP73 or I-9 ELISA's were expressed as relative absorbance values (mean ± SD, n=3) with absorbance of the respective positive controls (sera of mice immunized with either II-1, TTP73 or I-9) set as 1.

ELISA to identify anti-II-1, anti-TTP73 and anti-I-9 antibodies that inhibit the binding of respectively anti-spacer autoantibodies II-1, TTP73 or I-9 to ADAMTS13

ELISA to determine the effective concentration at half maximal binding (EC50) of each anti-spacer autoantibody to ADAMTS13

The EC50 of the anti-spacer autoantibodies II-1, TTP73 and I-9 was determined via ELISA. A 96-well microtiter plate was coated with recombinant human (rh)ADAMTS13 (15nM in PBS) and incubated overnight at 4°C. After blocking, the anti-spacer autoantibodies II-1, TTP73 or I-9 were added (10 µg/mL) and a 1 in 2 dilution series was made. Bound anti-spacer autoantibodies II-1, TTP73 or I-9 were detected using HRP-labelled rabbit anti-human IgG and IgM antibodies (1/10,000; Jackson ImmunoResearch). Colouring reaction was performed as described above and absorbance was measured at 490 nm. Binding curves were fitted using specific binding with Hill slope to determine EC50 for each anti-spacer autoantibody (Graphpad Prism v5.03 software Inc., San Diego, CA, USA). The determined EC50 for anti-spacer autoantibody II-1, TTP73 and I-9 are respectively 0.04, 0.85 and 0.04 µg/mL.

ELISA to identify inhibiting anti-II-1, anti-TTP73 and anti-I-9 antibodies

Human anti-spacer autoantibodies II-1, TTP73 or I-9 (constant final EC50) were pre-incubated with a 1 in 2 dilution of murine anti-II-1, anti-TTP73 or anti-I-9 antibodies (final start concentration 10 µg/mL) respectively, in a pre-blocked plate. After 30 minutes, samples were transferred to a rhADAMTS13 (15nM in PBS) coated 96-well microtiter plate. Bound human anti-spacer autoantibodies II-1, TTP73 or I-9 were detected using a mixture of HRP-labelled anti-human $IgG_{1\text{-}4}$ (IgG_1: 1/20,000 and $IgG_{2\text{-}4}$: 1/2,000; Sanquin, Amsterdam, The Netherlands). Colouring reaction was performed as described above and absorbance was measured at 490 nm. Binding (%) of anti-spacer autoantibodies II-1, TTP73 or I-9 to rhADAMTS13 in the presence of the competing murine anti-II-1 (17H9), anti-TTP73 (9G12) or anti-I-9 (7D10) antibody was expressed relative to binding of respectively II-1, TTP73 or I-9 with no competing antibody (buffer only) to rhADAMTS13 (set at 100% binding). Data were expressed as mean ± SD (n=3).

ELISA to study the binding of the anti-idiotypic antibodies to the anti-spacer idiotopes of II-1, TTP73 and I-9

Murine anti-idiotypic antibodies 17H9 (anti-II-1 antibody), 9G12 (anti-TTP73 antibody) and 7D10 (anti-I-9 antibody) (5 µg/mL in carbonate/bicarbonate coating buffer) were coated on 96-well microtiter plates. After blocking, human anti-spacer autoantibodies II-1, TTP73 and I-9 were added at a start concentration of 1 µg/mL and 1 in 2 serial diluted. Addition of a pool of human Immunoglobulin G (IgG) antibodies (Sigma-Aldrich Saint-Louis, MO, USA) was included as a negative control. Bound anti-spacer autoantibodies were detected by adding a mixture of HRP-labelled anti-human $IgG_{1\text{-}4}$ antibodies (IgG_1: 1/20,000 and $IgG_{2\text{-}4}$: 1/2,000; Sanquin). Colouring reaction was performed as described above and absorbance was measured

at 490 nm. Data were expressed as relative absorbance values (mean ± SD, n=3) with absorbance of binding of II-1, TTP73 and I-9 at 1 µg/mL to anti-idiotypic antibodies 17H9 (anti-II-1 antibody), 9G12 (anti-TTP73 antibody) and 7D10 (anti-I-9 antibody) respectively set as 1.

Patient samples

Plasma samples were collected from 151 iTTP patients during acute phase before treatment. All patients presented with TTP-related clinical signs (thrombocytopenia, microangiopathic haemolytic anaemia), ADAMTS13 activity <10% measured via FRETS-VWF73 assay (Peptides International, Louisville, KY, USA)[8] with exception of 2 samples that were measured using the collagen binding assay (CBA, patient's ID 128 and 131, Supplemental Table 1 indicated by *)[9] and anti-ADAMTS13 IgG titer >15 IU/mL measured via the TECHNOZYM ADAMTS13-INH ELISA® kit (Technoclone, Vienna, Austria) with exception of 3 samples that were measured using an in house anti-ADAMTS13 IgG ELISA (patient's ID 124, 128 and 129, Supplemental Table 1 indicated by §). Patients were diagnosed with idiopathic iTTP (without any underlying cause). Twenty-one plasma samples were derived from the University Medical Center Utrecht (The Netherlands), 35 samples from the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center of Milan (Italy) and 95 plasma samples from the French Reference Center for Thrombotic MicroAngiopathies (TMA) (France).
Besides age, sex, total anti-ADAMTS13 IgG titer and ADAMTS13 activity, detailed information on laboratory, clinical and outcome parameters was available for the 95 iTTP patients enrolled in the French Reference Center for TMA (Supplemental Table 2). Laboratory parameters such as platelet count and lactate dehydrogenase (LDH) levels (except for 10 patients) were available (Supplemental Table 2). Assessment of cerebral involvement at time of diagnosis included clinical signs including headaches, confusion, aphasia, transient focal defects, convulsion, seizure, stroke and/or coma. Treatment consisted of either plasma exchange (PEX) with/without rituximab, or PEX with/without rituximab supplemented with additional treatment(s); steroids, other immunosuppressive drugs (e.g. cyclophosphamide, bortezomib) and/or caplacizumab and/or splenectomy. Patient's outcome was investigated in terms of the pre-defined scoring system established by Benhamou *et al*. which includes age (years), LDH level (elevated LDH level: ≥ 2500 IU/L) and cerebral involvement.[10]

ELISA to identify the presence of anti-spacer idiotope profiles in plasmas of acute iTTP patients using the newly developed anti-idiotypic antibodies

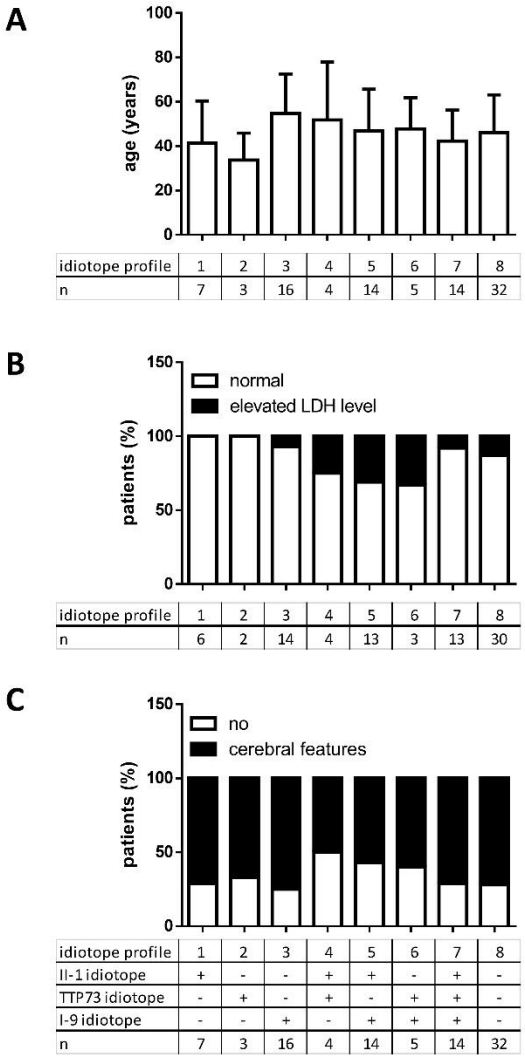
Ninety-six-well microtiter plates were coated with either murine anti-idiotypic antibody 17H9 (anti-II-1 antibody), 9G12 (anti-TTP73 antibody) or 7D10 (anti-I-9 antibody) (5 µg/mL in carbonate/bicarbonate

coating buffer) and incubated overnight at 4°C. After blocking, patient plasma (start dilution 10%, v/v) was added and 1 in 2 serial diluted. Bound patient antibodies (antibodies with the same idiotopes as the anti-spacer autoantibodies II-1, TTP73, or I-9), were detected with HRP-labelled anti-human IgG_{1-4} (IgG_1: 1/20,000 and IgG_{2-4}: 1/2,000; Sanquin). Colouring reaction was performed as described above and absorbance was measured at 490 nm. The human anti-spacer autoantibodies II-1, TTP73 or I-9 were used to set up a calibration curve. Anti-spacer autoantibody II-1 (0.25 µg/mL), TTP73 (1.25 µg/mL) or I-9 (0.25 µg/mL) were spiked in a normal human plasma pool of 10 healthy donors (NHP; start dilution 10%, v/v) and 1 in 2 serial diluted. The equation derived after linear regression was used to determine respectively II-1, TTP73 or I-9 idiotope levels (ng/mL) in patient samples. As a negative control, NHP was added in each assay in triplicate and used to define the Lower Limit of Detection (LLoD, mean of negative control + 3*SD) for the II-1, TTP73 and I-9 idiotope screening ELISA ($LLoD_{II\text{-}1\ idiotope}$ = 0.8 ng/mL, $LLoD_{TT773\ idiotope}$= 3.9 ng/mL and $LLoD_{I\text{-}9\ idiotope}$ = 0.8 ng/mL).

Statistical analysis

Graphpad Prism v5.03 software (GraphPad Software Inc.) was used for statistical analysis. Continuous variables were described as mean and standard deviation (SD) and categorical variables as counts and percentages. Continuous (age) and categorical (categorized LDH levels, cerebral involvement, score system by Benhamou *et al*.[10] and treatment) data were compared using ANOVA and chi square tests, respectively.

Supplemental Figure + Figure Legends



idiotope profile	1	2	3	4	5	6	7	8
n	7	3	16	4	14	5	14	32

idiotope profile	1	2	3	4	5	6	7	8
n	6	2	14	4	13	3	13	30

idiotope profile	1	2	3	4	5	6	7	8
II-1 idiotope	+	-	-	+	+	-	+	-
TTP73 idiotope	-	+	-	+	-	+	+	-
I-9 idiotope	-	-	+	-	+	+	+	-
n	7	3	16	4	14	5	14	32

Supplemental Figure 1: Separate parameters included in the score by Benhamou *et al.*[10] according to the anti-spacer idiotope profiles Stratification of the 95 acute iTTP patients of the French Reference Center for TMA according to the 8 idiotope profiles for (A) age, (B) lactate dehydrogenase (LDH) levels (normal levels, white bars; elevated levels: ≥ 2500 IU/L, black bars) and (C) cerebral involvement (no cerebral involvement, black bars; cerebral involvement, white bars). Ten patients were excluded since no LDH measurement was performed (Supplemental Table 2, indicated by N/A).

Supplemental Tables + Table Legends

Supplemental Table 1: ADAMTS13 activity, anti-ADAMTS13 IgG, anti-spacer II-1, anti-spacer TTP73 and anti-spacer I-9 idiotopes of iTTP patients during acute TTP Identity (ID), ADAMTS13 activity (%) using FRETS-VWF73 assay or collagen binding assay (*), anti-ADAMTS13 IgG titer (IU/mL) via TECHNOZYM® or in-house anti-ADAMTS13 IgG ELISA (§), anti-spacer II-1 idiotope, anti-spacer TTP73 idiotope and anti-spacer I-9 idiotope levels (ng/mL) of 151 acute iTTP patients. 'x' indicates no detectable anti-spacer idiotopes.

ID	ADAMTS13 activity (%)	anti-ADAMTS13 IgG (IU/mL)	anti-spacer II-1 idiotope (ng/mL)	anti-spacer TTP73 idiotope (ng/mL)	anti-spacer I-9 idiotope (ng/mL)
1	< 5	56	75	x	70
2	< 5	78	29	x	x
3	< 5	22	x	x	121
4	< 10	18	49	556	70
5	< 5	100	49	x	91
6	< 5	100	x	x	x
7	< 5	100	x	x	x
8	< 5	64	x	x	x
9	< 5	100	x	x	x
10	< 5	100	144	412	146
11	< 5	100	x	x	x
12	< 5	31	19	x	120
13	< 5	100	x	x	44
14	< 5	57	x	x	x
15	< 5	85	x	x	x
16	< 5	100	141	x	x
17	< 5	100	x	x	x
18	< 5	98	x	x	x
19	< 5	29	x	x	x
20	< 5	100	x	x	x
21	< 5	100	x	x	x
22	< 5	100	38	x	26
23	< 5	57	x	x	24
24	< 5	29	x	116	x
25	< 5	100	75	165	71
26	< 5	90	x	x	x
27	< 5	26	26	147	88
28	< 5	59	37	392	38
29	< 5	56	53	122	62

30	< 5	100	x	x	125
31	< 5	100	52	133	x
32	< 5	100	124	439	83
33	< 5	100	19	916	x
34	< 5	100	23	x	x
35	< 5	100	x	394	x
36	< 5	68	x	x	x
37	< 5	58	191	333	x
38	< 5	69	35	124	x
39	< 5	69	x	x	355
40	< 5	32	5	x	22
41	< 5	100	x	x	x
42	< 5	45	31	x	34
43	< 5	77	x	162	28
44	< 5	100	x	x	31
45	< 5	52	x	156	16
46	< 5	100	x	x	x
47	< 5	100	x	x	14
48	< 5	100	69	87	96
49	< 5	27	x	x	x
50	< 5	67	29	x	x
51	< 5	38	22	x	x
52	< 5	80	x	x	x
53	< 5	87	x	x	x
54	< 5	100	x	x	x
55	< 5	100	x	x	7
56	< 5	87	x	x	x
57	< 5	100	74	109	635
58	< 5	100	102	x	77
59	< 5	100	18	x	59
60	< 5	100	x	x	63
61	< 5	100	164	359	345
62	< 5	44	28	196	118
63	< 5	76	43	x	157
64	< 5	100	119	174	132
65	< 5	60	27	x	45
66	< 10	24	x	x	x
67	< 5	58	x	x	x
68	< 5	18	x	x	259
69	< 5	100	x	746	38
70	< 5	89	x	x	83
71	< 5	65	16	x	90
72	< 5	36	23	x	45

73	< 5	44	x	x	59
74	< 5	100	x	114	35
75	< 10	100	x	x	40
76	< 5	100	16	54	47
77	< 5	24	x	x	45
78	< 10	18	29	51	75
79	< 5	100	57	x	52
80	< 5	100	x	x	x
81	< 5	80	x	x	113
82	< 5	100	x	65	47
83	< 5	100	x	x	x
84	< 10	28	x	x	x
85	< 5	100	x	x	49
86	< 5	100	x	x	x
87	< 5	100	x	x	x
88	< 5	100	x	x	x
89	< 10	42	25	x	x
90	< 5	100	x	x	x
91	< 5	90	x	x	x
92	< 5	42	x	416	x
93	< 5	100	56	x	66
94	< 5	17	x	x	x
95	< 5	45	68	x	x
96	< 10	100	x	x	x
97	< 5	100	81	39	57
98	< 5	100	x	20	x
99	< 10	100	x	x	46
100	< 5	100	x	x	x
101	< 5	100	x	x	16
102	< 5	100	79	259	387
103	< 10	100	x	x	x
104	< 5	77	45	x	40
105	< 10	77	x	x	22
106	<10	76	x	x	x
107	< 5	74	x	x	x
108	< 10	65	x	x	x
109	< 5	62	x	x	x
110	< 5	60	40	572	x
111	< 10	58	x	x	x
112	< 5	58	x	338	x
113	< 10	58	x	x	x
114	< 5	54	x	x	27
115	< 5	50	13	293	43

116	< 5	100	59	403	50
117	< 5	18	x	x	x
118	< 5	100	x	x	x
119	< 5	33	x	x	x
120	< 5	16	x	x	54
121	< 5	100	x	x	x
122	< 10	26	x	388	x
123	< 5	100	x	x	x
124	< 5	39[§]	x	x	x
125	< 5	100	x	x	x
126	< 5	42	x	x	x
127	< 5	92	41	x	x
128	< 6*	11[§]	x	593	781
129	< 5	3.4[§]	x	x	x
130	< 5	86	22	153	x
131	< 6*	100	x	x	x
132	< 5	68	x	x	47
133	< 5	80	54	24	x
134	< 5	36	x	x	x
135	< 5	100	x	x	x
136	< 5	100	x	x	x
137	< 5	100	x	x	40
138	< 5	93	x	x	x
139	< 5	100	61	x	x
140	< 5	35	x	x	x
141	< 5	100	x	x	x
142	< 5	100	x	x	x
143	< 5	85	x	x	x
144	< 5	76	x	x	x
145	< 5	56	x	x	171
146	< 5	100	x	x	x
147	< 5	100	x	x	x
148	< 5	29	56	x	x
149	< 5	63	x	x	x
150	< 5	54	114	x	x
151	< 5	31	101	x	x

Supplemental Table 2: Detailed information on laboratory, clinical and outcome parameters of the 95 iTTP patients of the French reference center. Identity (ID; patients 1-95 depicted here, are the same patients 1-95 in Table 1), sex (M: male, F: female), age (years), platelet count (x10^9/L), idiotope profile (1-8), Benhamou score (< 3 (1-2) or ≥ 3 (3-4)), lactate dehydrogenase levels (LDH) level (IU/L), cerebral involvement and treatment. N/A: not assessed, † deceased, PEX: plasma exchange

ID	sex (M/F)	age (years)	platelet count (x10^9/L)	idiotope profile	Score by Benhamou *et al.*[40]	LDH level (IU/L)	cerebral involvement	additional treatment to PEX
1	F	66	24	5	≥ 3 (3)	< 2500	yes (seizure, headaches, confusion)	+ rituximab + steroids
2	F	41	8	1	< 3 (2)	N/A	yes (confusion)	PEX only
3	M	56	17	3	< 3 (2)	< 2500	yes (stroke)	+ steroids
4	F	65	132	7	≥ 3 (3)	< 2500	yes (confusion, transient focal defect)	+ rituximab + steroids
5	F	74	16	5	≥ 3 (3)	< 2500	yes (stroke)	PEX only
6	M	55	52	8	< 3 (1)	< 2500	no	+ rituximab + steroids + cyclophosphamide
7	M	74	41	8	≥ 3 (3)	< 2500	yes (confusion)	+ rituximab + steroids
8	M	33	6	8	< 3 (1)	< 2500	yes (headaches, convulsion and seizure)	+ rituximab + steroids
9	F	45	4	8	< 3 (2)	≥ 2500	no	steroids only
10	F	27	16	7	< 3 (1)	< 2500	yes	+ steroids
11	M	55	24	8	< 3 (1)	N/A	no	no treatment†
12	F	27	7	5	< 3 (0)	< 2500	no	+ steroids
13	M	68	23	3	≥ 3 (3)	< 2500	yes	+ rituximab + steroids
14	F	25	13	8	< 3 (1)	< 2500	yes (headaches)	+ rituximab + steroids
15	F	48	9	8	< 3 (2)	< 2500	yes (headaches)	+ rituximab + steroids
16	F	31	18	1	< 3 (1)	< 2500	yes (confusion)	PEX only
17	F	37	32	8	< 3 (1)	< 2500	yes (headaches)	PEX only

18	M	30	21	8	< 3 (0)	< 2500	no	+ rituximab + steroids + cyclophosphamide + bortezomib
19	F	65	14	8	≥ 3 (3)	< 2500	yes (transient focal defect)	+ steroids
20	F	60	18	8	< 3 (2)	≥ 2500	no	+ rituximab
21	M	48	26	8	< 3 (2)	< 2500	yes (confusion)	+ rituximab
22	F	56	27	5	< 3 (2)	< 2500	yes (stroke, blindness)	+ steroids
23	F	32	13	3	< 3 (1)	< 2500	yes (aphasia)	+ rituximab + steroids
24	F	23	12	2	< 3 (0)	< 2500	no	+ rituximab + steroids
25	M	38	16	7	< 3 (1)	< 2500	yes (headaches)	+ caplacizumab
26	F	26	7	8	< 3 (1)	< 2500	yes (headaches)	+ rituximab + steroids
27	M	34	44	7	< 3 (1)	< 2500	yes	PEX only
28	F	62	9	7	≥ 3 (3)	≥ 2500	yes (aphasia)	PEX only
29	M	24	18	7	< 3 (0)	< 2500	no	PEX only
30	F	38	17	3	< 3 (0)	< 2500	no	PEX only
31	F	80	14	4	≥ 3 (3)	< 2500	yes (confusion, convulsion)	+ rituximab
32	M	44	20	7	< 3 (2)	< 2500	yes (confusion, headaches)	PEX only
33	F	31	5	4	< 3 (0)	< 2500	no	+ steroids
34	F	71	20	1	≥ 3 (3)	< 2500	yes (convulsion)	PEX only
35	F	31	9	2	< 3 (1)	< 2500	yes (headaches)	+ rituximab
36	F	27	20	8	< 3 (1)	N/A	yes (headaches)	+ steroids
37	F	28	5	4	< 3 (1)	≥ 2500	no	+ steroids
38	F	68	5	4	≥ 3 (3)	< 2500	yes (seizure)	+ rituximab + steroids
39	F	45	39	3	< 3 (2)	< 2500	yes (transient focal defect)	+ rituximab + steroids
40	F	21	14	5	< 3 (2)	≥ 2500	yes (confusion, headaches)	+ steroids
41	F	63	12	8	< 3 (2)	< 2500	no	steroids only
42	M	35	7	5	< 3 (0)	< 2500	no	+ rituximab

43	F	41	12	6	< 3 (2)	N/A	yes (headaches)	+ steroids
44	F	53	31	3	< 3 (1)	N/A	no	+ rituximab
45	F	35	12	6	< 3 (2)	≥ 2500	yes (headaches, visual disorders)	+ steroids
46	F	54	10	8	< 3 (2)	< 2500	yes (transient focal defect)	+ steroids
47	M	58	13	3	< 3 (2)	< 2500	yes (confusion)	+ rituximab
48	F	24		7	< 3 (1)	< 2500	yes (aphasia)	+ rituximab + steroids
49	F	40	10	8	< 3 (1)	< 2500	yes (confusion)	+ rituximab + steroids
50	F	65	12	1	< 3 (2)	< 2500	no	+ rituximab + steroids
51	F	23	6	1	< 3 (1)	< 2500	yes (headaches)	+ steroids
52	M	20	9	8	< 3 (1)	< 2500	yes (headaches, convulsion)	+ rituximab + steroids
53	M	52	4	8	< 3 (1)	< 2500	no	+ rituximab + steroids + vincristin + splenectomy (anti-vWF Abs)
54	F	76	41	8	≥ 3 (3)	< 2500	yes (stroke)	+ rituximab
55	F	53	12	3	≥ 3 (3)	≥ 2500	yes (confusion, convulsion)	+ rituximab + steroids
56	F	70	30	8	≥ 3 (3)	< 2500	yes (stroke)	PEX only
57	M	41	18	7	< 3 (1)	< 2500	no	PEX only
58	F	66	26	5	≥ 3 (4)	≥ 2500	yes (confusion, stroke)	+ rituximab + steroids
59	F	25	12	5	< 3 (1)	≥ 2500	no	+ steroids
60	M	32	10	3	< 3 (1)	< 2500	yes	+ rituximab + steroids
61	F	53	5	7	< 3 (2)	< 2500	yes (disorder of language)	+ steroids
62	F	41	13	7	< 3 (1)	< 2500	no	PEX only
63	M	36	9	5	< 3 (0)	N/A	no	PEX only
64	F	34	8	7	< 3 (1)	< 2500	yes	+ rituximab + steroids
65	F	50	18	5	< 3 (2)	< 2500	yes	+ rituximab + steroids

66	F	45	11	8	< 3 (2)	< 2500	yes (headaches, visual disorders)	PEX only
67	M	60	6	8	≥ 3 (3)	≥ 2500	yes (headaches)	+ steroids
68	F	79	82	3	≥ 3 (3)	< 2500	yes (confusion, coma)	+ rituximab
69	M	65	35	6	≥ 3 (3)	< 2500	yes (aphasia, confusion)	+ steroids
70	M	27	29	3	< 3 (1)	< 2500	yes (headaches)	+ steroids
71	F	45	6	5	< 3 (1)	< 2500	no	+ steroids
72	F	67	8	5	≥ 3 (3)	≥ 2500	no	+ rituximab + steroids
73	M	44	32	3	< 3 (1)	N/A	no	PEX only
74	F	37	13	6	< 3 (0)	< 2500	no	+ steroids
75	M	61	13	3	< 3 (2)	< 2500	no	PEX only
76	M	42	10	7	≥ 3 (3)	< 2500	yes (stroke)	+ rituximab + steroids
77	F	83	12	3	≥ 3 (3)	< 2500	yes (stroke)	PEX only
78	M	63	30	7	< 3 (2)	N/A	no	PEX only
79	F	64	4	5	≥ 3 (3)	< 2500	yes (headaches, transient focal defect)	+ rituximab + steroids
80	F	85	9	8	≥ 3 (3)	< 2500	yes (transient focal defect)	+ steroids
81	M	83	9	3	≥ 3 (3)	< 2500	yes (confusion)	+ rituximab + steroids
82	M	61	11	6	< 3 (2)	N/A	no	+ rituximab + steroids + cyclophosphamide + splenectomy
83	F	33	63	8	< 3 (1)	< 2500	yes (transient focal defect)	steroids only
84	M	40	34	8	< 3 (1)	< 2500	yes (cerebral lesions)	+ steroids
85	F	63	7	3	≥ 3 (3)	< 2500	yes (stroke)	no treatment†
86	F	40	8	8	< 3 (1)	< 2500	yes (headaches)	+ steroids
87	F	27	11	8	< 3 (0)	< 2500	no	+ steroids
88	F	21	11	8	< 3 (1)	< 2500	yes (headaches, transient focal defect)	+ rituximab

89	F	31	55	1	< 3 (1)	< 2500	yes (headaches, seizure)	+ rituximab + steroids
90	F	33	6	8	< 3 (1)	< 2500	yes (headaches)	+ rituximab + steroids
91	F	46	5	8	≥ 3 (3)	≥ 2500	yes (headaches)	+ rituximab + steroids
92	M	47	10	2	< 3 (2)	N/A	yes (seizure, aphasia, coma)	no treatment†
93	M	24	7	5	< 3 (1)	< 2500	yes (headaches)	+ rituximab + steroids
94	M	44	7	8	< 3 (1)	< 2500	no	+ rituximab + steroids
95	F	27	8	1	< 3 (0)	< 2500	no	+ rituximab + steroids

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