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Thrombotische Mikroangiopathien, speziell die thrombotisch thrombozytopenische Purpura und die Metalloprotease ADAMTS13

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Abkürzungsverzeichnis

Abkürzung	Erläuterung
ADAMTS13	a disintegrin and metalloprotease with thrombospondin-type 1 repeats number 13
COVID-19	Coronavirus Erkrankung 2019
CRP	C-reaktives Protein
DIC	Dissemierte intravasale Gerinnung (Verbrauchskoagulopathie)
FFP	Fresh Frozen Plasma
FLei	Fragebogen zur subjektiven Einschätzung der geistigen Leistungsfähigkeit
GAD-7	Generalized Anxiety Disorder 7
Hb	Hämoglobin
HIT	Heparin-induzierte Thrombozytopenie
HMWM	Hochmolekulare (VWF) Multimere
HUS	Hämolytisch urämische Syndrom
IDS-SR	Inventory of Depressive Symptomatology, Self-Report
IL-6	Interleukin 6
IL-8	Interleukin 8
IMWM	VWF Multimere von intermediärem Molekulargewicht
ITP	Immunthrombozytopenie
iTTP	(auto-)immune thrombotisch thrombozytopenische Purpura
MAHA	mikroangiopathische hämolytische Anämie
NS	Nicht signifikant
LMWM	Nierdmolekulare (VWF) Multimere
LDH	Lactatdehydrogenase
LOT-R	Life Orientation Test–Revised
LTX	Lebertransplantation
PEX	Plasmaaustausch/ Plasmapherese
PHQ-9	Patient Health Questionnaire 9 Items
QLQ-C30	Quality of Life Questionnaire C 30
RS-11	Resilienz Skala 11

TMA	Thrombotische Mikroangiopathie
TNF-α	Tumornekrosefaktor alpha
TTP	Thrombotisch thrombozytopenische Purpura
UL VWF M	Ultralange Von Willebrand Faktor Multimere
VWF	Von Willebrand Faktor
VWF MM Ratio	Von Willebrand Faktor Multimer Ratio

1. Einleitung

Die thrombotischen Mikroangiopathien (TMA) sind eine ätiologisch sehr heterogene Gruppe, die unter anderem das hämolytisch urämische Syndrom (HUS), die thrombotische thrombozytopenische Purpura (TTP) und weitere sekundäre thrombotische Mikroangiopathien umfasst (1). Ihnen allen gemein ist eine hämolytische Anämie und Thrombozytopenie, wodurch sich die Abgrenzung der einzelnen TMAs als schwierig gestaltet (Abb.1).

Klinisch ist dem HUS eher die Nierenbeteiligung zuzusprechen und die neurologischen Symptome der TTP, aber dies ist trügerisch, da es auch hier Überlappungen geben kann und eine eindeutige Zuordnung nicht immer möglich ist. Darüber hinaus zeigen einige Erkrankungen typische TMA Anzeichen, sind aber einer anderen Grunderkrankung zuzuordnen. Mittels spezifischer und stufenweiser Diagnostik kann der Ursache auf den Grund gegangen werden (Abb.1).

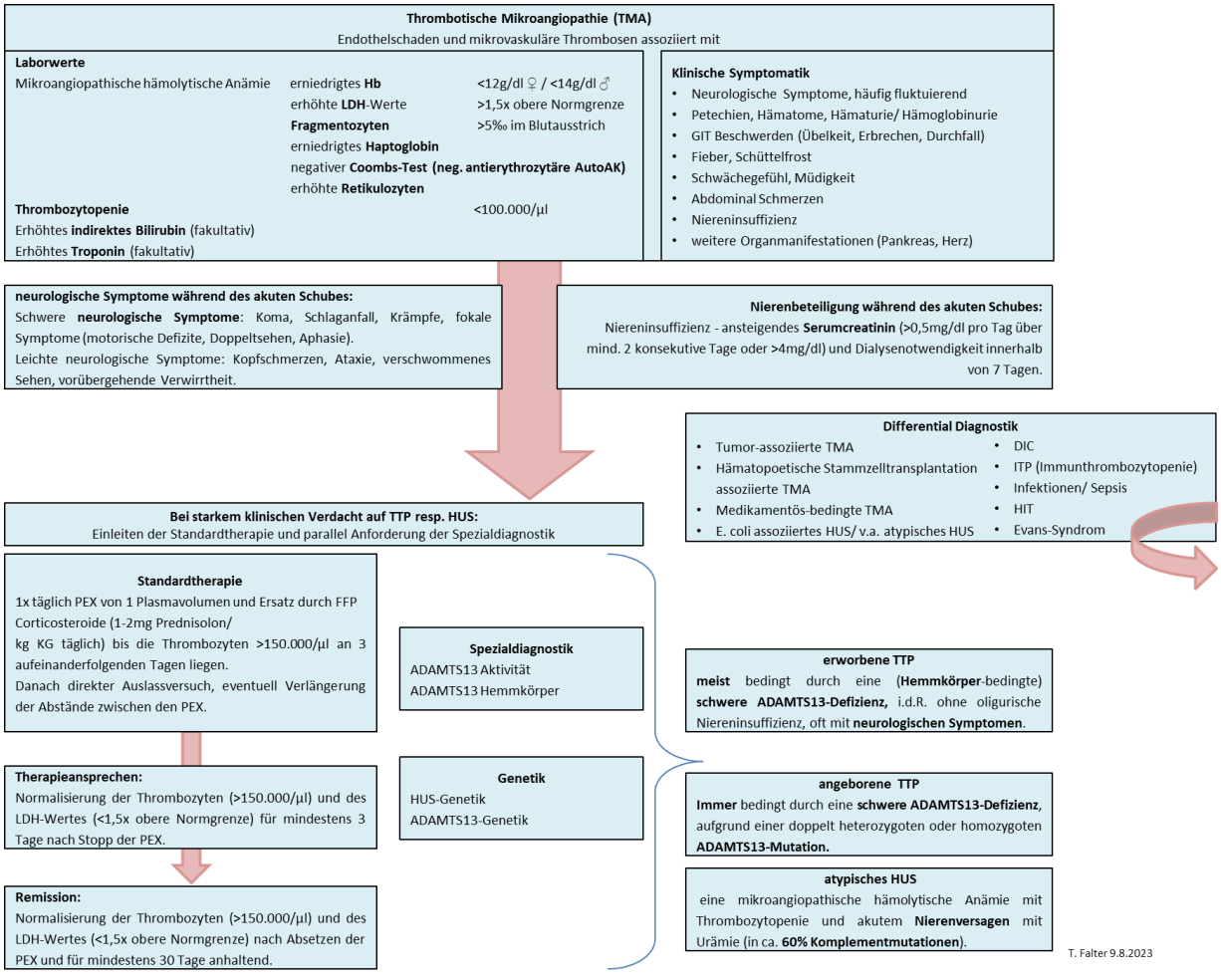


Abb.1: Flussdiagramm zur Differentialdiagnostik der TMAs.

1.1. Thrombotisch thrombozytopenische Purpura (TTP)

Bei der thrombotisch thrombozytopenischen Purpura (TTP) handelt es sich um eine potentiell lebensbedrohliche Erkrankung, die in Form akuter, oft rezidivierender Schübe auftritt. Mit einer jährlichen Inzidenz von ca. 2 bis 6 Fällen pro 10^6 Einwohner handelt es sich um eine seltene Erkrankung, die den thrombotischen Mikroangiopathien zugeordnet wird (2).

Ein akuter TTP Schub ist gekennzeichnet durch das Auftreten von Von Willebrand Faktor (VWF)– Thrombozyten-reichen Mikrothromben in Arteriolen und Kapillaren (Abb. 2) (3). In der Folge kommt es zu Ischämien unterschiedlicher Intensität des jeweils betroffenen Organs. Charakteristischerweise ist bei der TTP das Gehirn betroffen mit damit einhergehenden neurologischen Auffälligkeiten wie Kopfschmerzen, Vigilanzstörungen, Koma, Krampfanfällen und fokalen Ausfällen. Darüber hinaus können aber auch eine renale Dysfunktion, kardiale Symptomatik und Ischämien anderer Organe wie Milz, Pankreas oder Nebennieren auftreten (4).

Durch den Verbrauch der Thrombozyten in den VWF-Thrombozyten—reichen Mikrothromben und aufgrund der mechanischen Zerstörung der Erythrozyten (Abb. 2) in der verlegten Endstrombahn, zeigt sich bei den Patienten eine Thrombozytopenie, sowie eine hämolytische Anämie mit Fragmentozyten.

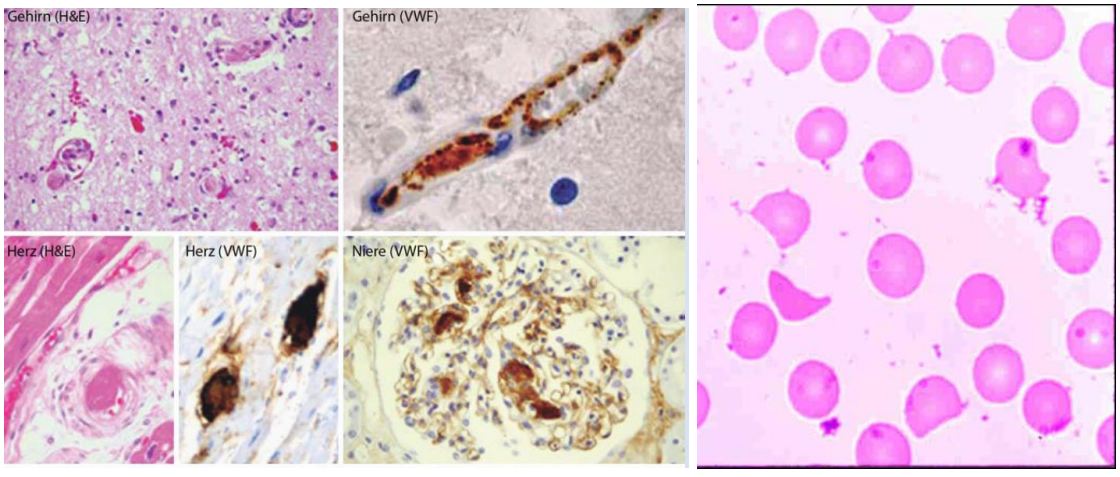


Abb. 2: Histologische Schnitte verschiedener Organe eines TTP Patienten mit VWF-Thrombozyten-reichen Mikrothromben (links) (3, 4). Fragmentozyten eines TTP Patienten im Blutausstrich.

Die erste Beschreibung einer thrombotischen thrombozytopenischen Purpura erfolgte bereits 1924 durch Eli Moschkowitz. Er beschrieb ein 16jähriges Mädchen, welches innerhalb von zwei Wochen nach dem abrupten Auftreten von petechialen Blutungen, Blässe, Fieber, Lähmungen, Hämaturie und Koma verstarb. Die Autopsie zeigte zahlreiche mikrovaskuläre, hyaline Thromben, die in Kapillaren und Arteriolen vorzufinden waren.

Der ursächliche Auslöser dieser Erkrankung blieb lange unbekannt. Während Moschkowitz von einem stark agglutinierenden und hämolytischen „Gift“ ausging, entdeckte Moake et al. 1982 im Plasma von TTP Patienten hochmolekulare VWF Multimere, und ging vom Fehlen einer VWF-Depolymerase als Ursache aus (5). Furlan und Tsai entdeckten 14 Jahre später, 1996, zeitgleich die VWF-spaltende Protease (6, 7). Deren Mangel konnten Furlan et al. 1997 als Ursache der thrombotisch thrombozytopenischen Purpura ausmachen (8). Bereits 1 Jahr darauf konnten IgG Autoantikörper gegen die VWF-spaltende Protease identifiziert werden, die die Defizienz der Protease erklärten (9-11). Allerdings zeigte sich in großen retrospektiven Studien mit TTP Patienten, dass es neben den Patienten mit Autoantikörpern auch Patienten mit kompletter Enzymdefizienz gab ohne Autoantikörper. Das legte die Vermutung nahe, dass es auch eine genetische Komponente geben musste (10).

2001, nach der Reinigung und Charakterisierung ihrer N-terminalen Aminosäurensequenz, wurde die VWF-spaltende Protease als 13tes Mitglied einer Familie von Metalloproteasen zugeordnet (12-14). Ihre Bezeichnung als ADAMTS13 stammt von „**a** **d**isintegrin **a**nd **m**etalloprotease with **t**hrombo**s**pondin-type 1 repeats **13**“ (12-16).

1.2. ADAMTS13 und der VWF

Die VWF-spaltende Protease, ADAMTS13, wird hauptsächlich in den Sternzellen der Leber produziert und in aktiver Form ins Plasma sezerniert und verweilt dort mit einer Halbwertszeit von zwei bis drei Tagen (17). Allerdings sind auch andere Zellen, wie die renalen Podocyten, tubuläre Zellen, vaskuläre Endothelzellen und Thrombozyten in der Lage ADAMTS13 zu exprimieren, wenn auch in geringeren Mengen (18-20). Die Konzentration von ADAMTS13 im Blut beträgt 1 µg/ml (13).

Wie ADAMTS13 reguliert wird, ist nach wie vor nicht restlos aufgeklärt. Sowohl der VWF als Substrat von ADAMTS13, inflammatorische Cytokine wie Interleukin-6 (IL-6), als auch Thrombin, Plasmin und Leukozytenelastase scheinen einen Einfluss auf die Regulierung zu haben (21).

ADAMTS13 ist aus mehreren Proteindomänen, die von 29 Exons des ADAMTS13 Gens codiert werden, aufgebaut: Einem Signalpeptid (SP), einem Propeptid (PP), einer Metalloprotease-Domäne (MP), der Disintegrin-Domäne (Dis), einer „thrombospondin type 1 repeat"-Domäne (TSR1), einer cysteinreichen Domäne (Cys), einer Spacer-Domäne (S), sieben weiteren TSR1 (2 bis 8) und zwei CUB-Domänen (C1r/C1s, epidermaler Wachstumsfaktor, Knochenmorphogenetisches Protein) (4, 15). Die mature ADAMTS13 Einheit besteht aus 1353 Aminosäuren, hat jedoch statt dem zu erwartenden Molekulargewicht von 145 kD ein Molekulargewicht von 190 kD. Dies liegt unter anderem an der hohen Glykosylierung von ADAMTS13 (4, 15).

Der für die primäre Hämostase wichtige VWF wird in den Endothelzellen und den Megakaryozyten gebildet und in hochmolekularer Form in den Speicherorganellen der Endothelzellen, den Weibel-Palade bodies, gespeichert. Der überwiegende Anteil der hochmolekularen, stark adhäsiven, prothrombotischen VWF Multimeren wird erst nach Stimulation und Aktivierung des Endothels aus den Weibel-Palade bodies freigesetzt. Ein kleiner Anteil des gebildeten VWFs wird kontinuierlich sezerniert und zirkuliert in unterschiedlich großen Multimereinheiten im Plasma (22).

An Orten mit Endotheldefekten und hohen Scherkräften, wie sie bei Gefäßverletzungen in der Mikrozirkulation vorherrschen, binden sich die VWF Multimere an Kollagen oder andere Komponenten der subendothelialen Matrix und entfalten sich. Die Konformationsänderung bewirkt eine Freilegung der „aktivierten A1-Domänen" und Thrombozyten lagern sich via Glykoprotein Ib an den hochmolekularen VWF an (23).

Unter physiologischen Bedingungen wird der VWF im Blut von ADAMTS13 in kleinere und weniger adhäsive Moleküle gespalten. Die Spaltung des VWFs erfolgt dabei an der Peptidbindung Y1605-M1606 in der Domäne A2 im katalytischen Zentrum durch ADAMTS13 (23) (Abb. 3).

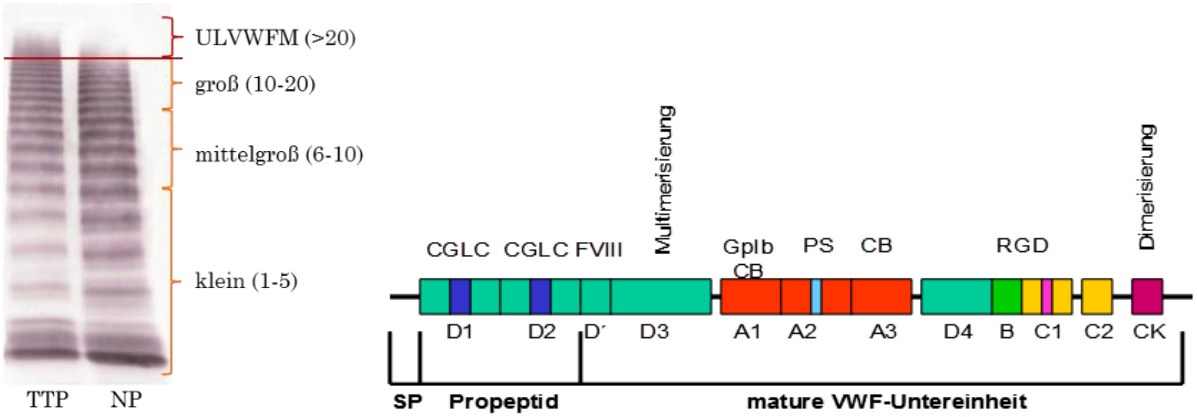


Abb. 3: (Links) Auftrennung der VWF Multimere im Plasma durch Elektrophorese und Detektion mittels Immunoblot (TTP Patientenplasma - TTP, Plasma eines gesunden Probanden - NP). (Rechts) Schematische Darstellung der Struktur des VWFs. SP: Signalpeptid, CGLC: Konsensus Sequenz von Disulfidisomerasen, FVIII: Bindungsstelle für Faktor VIII, GpIb: Bindungsstelle für Glykoprotein Ib-alpha, PS: proteolytische Spaltstelle von ADAMTS13, CB: Kollagenbindungsstelle, RGD-Sequenz: GpIIb/IIIb Bindungsstelle, CK: „cystin knot“ Domäne (nach Tsai HM. (4)).

1.3. Pathophysiologie der TTP

Bei Patienten mit thrombotisch thrombozytopenischer Purpura unterbleibt die Spaltung der hochmolekularen, prothrombotischen VWF Multimere in kleinere weniger adhäsive Moleküle aufgrund einer schweren Defizienz der ADAMTS13 (ADAMTS13-Aktivität <10%) (Abb. 4).

An Orten mit hohen Scherkräften, wie sie in Arteriolen und Kapillaren auftreten, bilden sich spontan Mikrothromben durch die Verklumpung des hochmolekularen VWF mit den Thrombozyten (4) (Abb. 4). In der Folge zeigen sich einerseits eine verbrauchsbedingte Thrombozytopenie, andererseits eine hämolytische Anämie aufgrund der mechanischen Zerstörung der Erythrozyten. Die Mikrothromben führen in den unterschiedlichsten Organen zu einer Ischämie mit den entsprechenden Ausfallerscheinungen. Daher ist die Symptomatik sehr variabel.

Führende klinische Symptome sind neurologische Auffälligkeiten. Angefangen bei milderen Symptomen wie Kopfschmerzen, Parästhesien, Fatigue, Sehstörungen und Schwindel bis hin zu schweren neurologischen Ausfällen wie Bewusstseinseintrübung, Aphasie, Paresen, Krampfanfällen und Koma. Beginnen kann ein akuter Schub aber auch mit Blutungszeichen wie Petechien, Hämatome, selten größeren Blutungen und

unspezifischen Symptomen wie Bauchschmerzen, Übelkeit, Erbrechen, Durchfall, Fieber, manchmal Brustschmerzen sowie Schwäche.

Ohne Behandlung der TTP kann es zu einer raschen Verschlechterung und zum Tod kommen. Die Letalität des akuten TTP Schubes ohne adäquate Therapie liegt bei 90% (24).

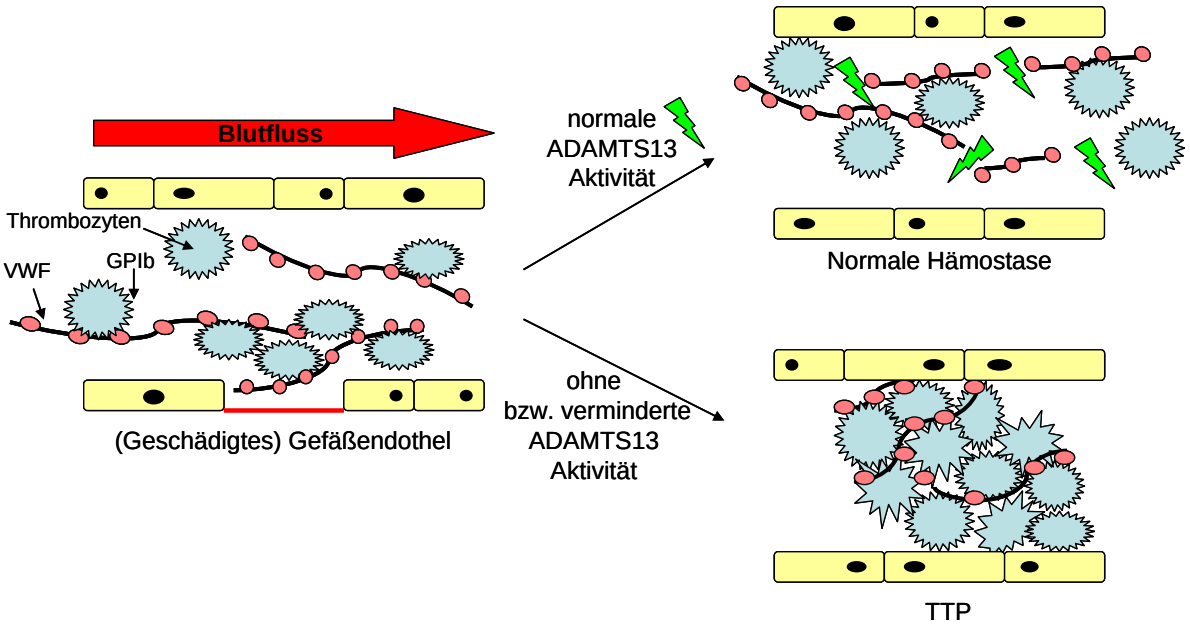


Abb. 4: Pathogenese der TTP: Der VWF wird in Form hochmolekularer Multimere aus dem Gefäßendothel ins Blut abgegeben und unter physiologischen Bedingungen in kleinere Multimere durch ADAMTS13 gespalten. Bei einem schweren ADAMTS13 Mangel kommt es an Orten mit hohem Scherstress in Anwesenheit des hochmolekularen VWF zu einer netzartigen Verlegung der Endstrombahn durch VWF- Thrombozyten- reiche Mikrothromben [nach Sadler (25)].

Im akuten Schub fällt die TTP labordiagnostisch durch eine Thrombozytopenie (<100.000/µl/, meist sogar <30.000/µl), eine Coombs-negative hämolytische Anämie mit erniedrigten Hämoglobinwerten, vermindertem Haptoglobin, sowie Fragmentozyten im Blutausstrich und erhöhten Lactatdehydrogenasewerten (LDH) auf. Bei notwendiger, weiterführender Diagnostik ist eine stark verminderte ADAMTS13-Aktivität (<10%) nachweisbar. Je nach TTP Form ist ein Inhibitor gegen ADAMTS13 oder eine Mutation im *ADAMTS13 Gen* auszumachen (26).

1.4. Formen der TTP

Die thrombotisch thrombozytopenische Purpura geht unweigerlich mit einem schweren Mangel der Protease ADAMTS13 einher. Die Ursache dieses Enzymmangels kann entweder durch einen Autoantikörper gegen ADAMTS13 bedingt sein oder aber an einer (homozygoten oder doppelt heterozygoten) Mutation im *ADAMTS13 Gen* liegen.

Je nach zugrundeliegendem Pathomechanismus kann somit in eine erworbene, autoimmune und eine hereditäre TTP (auch Upshaw-Schulman-Syndrom) eingeteilt werden, wobei die erworbene Form deutlich häufiger vorkommt als die hereditäre (1). Beide Formen können prinzipiell in jedem Alter klinisch manifest werden. Die Initialdiagnose der hereditären TTP wird allerdings meist in den ersten zwei Lebensjahrzehnten gestellt, die der erworbenen TTP zwischen dem 30. bis 60. Lebensjahr (27).

Bei der erworbenen TTP kann der Autoantikörper isoliert (idiopathische TTP) oder im Zusammenhang mit einer anderen Grunderkrankung wie einem systemischen Lupus Erythematodes, bestimmten Medikamenten (z. B. Ticlopidin) oder einer HIV-Infektion auftreten (1, 28), was dann oft als sekundäre immunologische TTP berichtet wird.

Bei den ADAMTS13 inhibierenden Autoantikörpern handelt es sich im Regelfall um Antikörper der Klasse IgG, die in erster Linie an die Spacer-Domäne, aber auch an andere Domänen von ADAMTS13 mit unterschiedlicher Ausprägung binden. Nachdem die Spacer-Region auch eine wichtige Bindungsstelle für die A2-Domäne des VWF darstellt, verursachen die Autoantikörper somit den Funktionsverlust der ADAMTS13-Aktivität. Neben der Inhibierung der Protease bewirkt die Bindung gewisser Autoantikörper an ADAMTS13 auch eine beschleunigte Clearance des Enzym-Antikörper-Komplexes (27, 29, 30).

Sowohl der inhibierende Autoantikörper als auch der damit verbundene ADAMTS13 Mangel können auch nach einem Schub fortbestehen, obwohl sich der Patient in klinischer Remission befindet. Daher werden weitere Triggermechanismen des akuten TTP Schubes vermutet. Dies ist für Autoimmunerkrankungen nicht ungewöhnlich. So sind die Schwangerschaft, aber auch schwere Infektionen und Stress sowohl als Auslöser einer Autoimmunerkrankung als auch als Trigger in der Diskussion (31, 32).

1.5. Therapie und weiterer Verlauf der TTP

Der akute TTP Schub ist mit einer hohen Sterblichkeit verbunden. Aus diesem Grund sind eine rasche und adäquate Diagnose und Therapie unerlässlich. Seit 1991 ist der Goldstandard der Therapie die Behandlung mittels Plasmaaustausch (PEX) und Glukokortikoiden. Damit konnte die Sterblichkeit von 90% auf rund 20% im akuten Schub verringert werden (24).

Ziel der PEX ist die Entfernung respektive Reduktion der ADAMTS13 Autoantikörper, sowie der hochmolekularen VWF Multimere und vor allem die gleichzeitige Zufuhr von aktivem ADAMTS13 durch Fresh Frozen Plasma (FFP).

Sobald der Patient sich klinisch stabilisiert hat und die Thrombozytenzahlen als auch der LDH Wert nahezu normal sind, kann die PEX unter klinischer Beobachtung pausiert werden (1). Insbesondere die Thrombozyten sind ein wichtiger Indikator in der Therapie – sie zeigen durch ihr Ansteigen sowohl das Therapieansprechen als auch durch ihren Abfall einen erneuten Krankheitsrückfall an.

Daneben hat sich der 1997 ursprünglich für das B-Zell-Non-Hodgkin Lymphom zugelassene chimäre, monoklonale anti-CD20-Antikörper, Rituximab als ein internationaler Therapiestandard bei der TTP entwickelt (1, 33). Rituximab unterdrückt die Produktion von ADAMTS13 Autoantikörper, indem es die B-Lymphozyten dezimiert (34). Trotz der weit verbreiteten Anwendung und Zulassung gegen einige andere Autoimmunerkrankungen wird Rituximab nach wie vor bei der TTP im off-label eingesetzt.

Seit 2020 steht mit Caplacizumab ein weiterer therapeutischer Antikörper bei der TTP zur Verfügung, der mittlerweile auch fester Bestandteil der empfohlenen Standardtherapie ist (35-40). Der zugelassene, humanisierte monoklonale Antikörper bindet an die A1 Domäne des VWFs und verhindert damit die Anlagerung an die Thrombozyten, wodurch die Bildung von Mikrothromben vermindert wird. Caplacizumab wird im akuten Schub direkt vor der ersten PEX intravenös verabreicht und danach bis 30 Tage nach Ende der PEX täglich subkutan gegeben (40).

Nach einem überstandenen akuten TTP Schub normalisieren sich die meisten Laborparameter, wie Thrombozyten, Hämoglobin, LDH etc., bei allen Patienten wieder. Die ADAMTS13-Aktivität steigt, bei gleichzeitigem Abfall der ADAMTS13-Autoantikörper, bei vielen TTP Patienten wieder an. Trotzdem gibt es auch einige in

klinischer Remission befindliche Patienten, die konstant niedrige ADAMTS13-Aktivitätswerte und nachweisbare Autoantikörper weiterhin aufweisen, was ein Risiko für ein Rezidiv einer klinisch manifesten TTP darstellt.

Nach einem initialen akuten Schub kommt es bei den Überlebenden in ca. 40% zu einem Rezidiv, weswegen man von einer chronisch rezidivierenden Erkrankung sprechen kann (41).

Aber nicht nur die potentiellen Rezidive, sondern auch langfristige Folgen der akuten Schübe sind mittlerweile in den Fokus gerückt, wenn auch nur wenig untersucht.

2. Fragestellungen, die im Rahmen der Habilitation erörtert wurden

Die vorliegende Habilitationsschrift befasst sich umfassend mit der autoimmunen thrombotisch thrombozytopenischen Purpura (iTTP), angefangen bei prädiktiven Markern eines akuten TTP Schubes, über den Schubverlauf bis hin zu den Langzeitfolgen der akuten Schübe. Darüber hinaus wird die Bedeutung der ADAMTS13 für die Abgrenzung der TTP gegenüber anderen Mikroangiopathien näher beleuchtet.

In einer ersten Aufarbeitung eines kleinen iTTP-Patiententeilkollektives wurden Krankheitsverlauf und potentielle Langzeitfolgen untersucht.

Aufgrund dieser Ergebnisse wurde eine umfangreiche prospektive Beobachtungsstudie über 4 Jahre initiiert. Über 100 iTTP-Patienten sind mittels validierter Fragebögen auf Depressionen, Angstzustände, ihre kognitive Leistungsfähigkeit, sowie die Lebensqualität und Lebenseinstellung hin untersucht worden.

In einer retrospektiven Beobachtungsstudie zu iTTP-Patienten sind die Schubhäufigkeit, die Schubschwere, sowie mögliche Einflussfaktoren auf die Schubfrequenz, auch im Hinblick auf neue Therapieoptionen wie Rituximab, untersucht worden.

Daran anschließend wurde ein vom Bundesministerium für Bildung und Forschung (BMBF) gefördertes prospektives iTTP Register angelegt mit dem Ziel prädiktive Marker für Morbidität und Mortalität des akuten Schubes und eines iTTP-Rezidivs zu identifizieren. In diesem Zuge wurden in Kooperation mit dem Laboratory of Thrombosis Research (KU Leuven Campus Kulak, Belgien) neuartige Analysen bezüglich der Konformation von ADAMTS13 vor, während und nach dem akuten Schub durchgeführt. Des Weiteren haben wir intensive Untersuchungen zu zahlreichen labordiagnostischen Parametern wie z.B. dem Komplementsystem vorgenommen.

Zudem stellt sich die Frage welche Rolle das Hauptsubstrat von ADAMTS13, der VWF, im Rahmen der iTTP spielt. Seine Menge, Aktivität und die Verteilung der VWF Multimere als auch potentielle Einflussfaktoren auf den VWF wurden ebenfalls eingehender betrachtet.

Darüber hinaus wurde eine aktuell relevante Erkrankung, bei der die Bildung von Mikrothromben eine Rolle zu spielen scheint, nämlich COVID-19, auf ADAMTS13 untersucht.

3. Methoden

Die Studien sind nach deutschem Recht [Landeskrankenhausgesetz §36 und §37] in Übereinstimmung mit der Deklaration von Helsinki und von der örtlichen Ethikkommission der Landesärztekammer Rheinland-Pfalz [837.265.14 (9504-F)], [837.506.15 (10274)], [2020-14988_2] genehmigt worden, und alle Teilnehmer haben ihre schriftliche Zustimmung zur Teilnahme gegeben.

Studientypen	**Anwendung in folgenden Originalarbeiten**
Retrospektive Beobachtungstudie	I, III, V
Prospektive Beobachtungsstudie	II, IV, VI
Validierte Fragebögen	
FLei	II, IV
IDS-SR	II
PHQ-9	IV
GAD-7	IV
RS-11	IV
LOT-R	IV
QLQ-C30	IV
Spezielle Labormethoden	
ADAMTS13-Aktivität	I - VI
ADAMTS13 Hemmkörper	I - VI
ADAMTS13 Autoantikörper*	VI
ADAMTS13 Antigen*	VI
ADAMTS13 Conformation*	VI
Ultralange VWF Multimer Gelelektrophorese	I
VWF Multimer Ratio (Sebia®)	VI
Mikroskopie von Fragmentozyten	I - VI

Statistische Methoden

Student´s t-Test	II, IV, VI
Mann-Whitney-U-Test	II, IV, V, VI
Wilcoxon-Test	II, IV
Kruskal-Wallis-Test	II
Rangkorrelation nach Spearman	II, IV
Korrelationskoeffzient nach Pearson	IV
Kaplan Meier Kurve / Log-Rank Test/ Cox Regression	III
Poisson-Modell	I, III
Andersen-Gill Modell	III
Chi-Quadrat-Test/ Fisher-Test	V

* diese Analytik wurde durchgeführt in KU Leuven Campus Kortrijk, Belgien, durch die Arbeitsgruppe von Prof. Karen Vanhoorelbeke

4. Ergebnisse und Diskussion

Nunmehr fast genau 100 Jahre nach ihrer Erstbeschreibung durch Moschkowitz ist die TTP nach wie vor nicht ausreichend erforscht. Natürlich ist es bei seltenen Erkrankungen nicht einfach, binnen kurzer Zeit zu großen Datenmengen und klaren Aussagen zu kommen. Es bedarf ausführlicher klinischer Beobachtungsstudien über längere Zeiträume, immer wieder neue Forschungsansätze und diagnostische, sowie therapeutische Möglichkeiten.

4.1. Untersuchungen zum Krankheitsverlauf und zur Charakteristik der akuten iTTP-Schübe, sowie zu prognostischen Markern des akuten Schubes.

4.1.1. Zugrundeliegende Patientenkollektive

Dem folgenden Abschnitt liegen vier verschiedene Patientenbeobachtungen zugrunde (**Originalarbeiten I, III, V und VI**).

1. Retrospektive Voruntersuchung, die die akuten iTTP-Schübe klinisch wie labormedizinisch charakterisiert, den Krankheitsverlauf und potentielle Schubauslöser beschreibt und die Langzeitfolgen näher betrachtet (**Originalarbeit I.**).

> In die Untersuchung gehen 21 iTTP-Patienten, 16 Frauen und 5 Männer, mit 103 akuten Schüben ein. Eingeschlossen wurden Patienten aus verschiedenen Kliniken, deren Krankengeschichte von der Initialdiagnose bis zum Studienzeitpunkt November 2012 vorhanden waren, und die alle in der Universitätsmedizin Mainz vorstellig geworden sind. Einschlusskriterium war zudem ein ADAMTS13-Aktivitätsmangel <5% und der Nachweis von ADAMTS13 Hemmkörpern, sowie mindestens ein akuter iTTP-Schub mit einer Thrombozytopenie und hämolytischen Anämie mit Fragmentozyten.
>
> Das Patientenkollektiv hatte vom Zeitpunkt der Erstdiagnose, mit durchschnittlich 25,5 Jahren (minimal 12, maximal 53 Jahre) bis zum Studienzeitpunkt durchschnittlich 4,8 Schübe (mindestens 1, maximal 12) erlitten. Die Beobachtungsspanne reicht dabei von einem bis hin zu 30 Jahren.

2. Retrospektive Beobachtungsstudie, anhand derer untersucht wurde, wie häufig akute iTTP-Schübe auftreten, welchen Schweregrad sie aufwiesen und inwieweit Rituximab einen Einfluss auf die Schubschwere und die Häufigkeit hat (**Originalarbeit III.**).

Wir haben in einer systematischen, retrospektiven Beobachtungsstudie alle iTTP-Patienten, die zwischen 2003 und 2014 an der Universitätsmedizin behandelt wurden oder konsiliarisch assoziiert waren, erfasst. 2003 wurde als Studienbeginn gewählt, da ab diesem Zeitpunkt Rituximab in off-label bei TTP Anwendung fand.

Bei 88 Patienten wurde klinische (Thrombozytopenie und hämolytischen Anämie) die Diagnose der TTP gestellt. Bei 70 Patienten konnte die Diagnose TTP aufgrund einer ADAMTS13 Defizienz gesichert werden. Bei 25 Patienten war die TTP Diagnose vor Beginn des Studienzeitraum, 2003, bekannt. 45 Patienten erhielten ihre Erstdiagnose nach 2003. Das mittlere Alter bei Erstdiagnose lag bei 33 Jahren. Die 70 Patienten, erlitten zusammen 224 akute Schübe, im Median 2 pro Person, über einen Beobachtungszeitraum von 8,3 Jahren (Spanne 0,4-31,9, IQR 4,3-14,3 Jahre). 150 akute Schübe bei 33 iTTP-Patienten wurden mittels Standardtherapie behandelt und 37 Patienten mit 69 akuten Schüben erhielten zusätzlich Rituximab. Die Daten von insgesamt 219 akuten Schüben waren komplett und somit für die Studie auswertbar.

3. Prospektive Beobachtungsstudie, die sich mit dem klinisch wie laborchemischen Verlauf des akuten Schubes, seiner Behandlung und potentiell prognostischen Markern beschäftigt (**Originalarbeit VI.**).

Von 91 potentiellen iTTP-Patienten im Zeitraum von Juli 2016 bis August 2018 wurden 83 iTTP-Patienten eingeschlossen und ihr Krankheitsverlauf klinisch wie laborchemisch prospektiv verfolgt. Zudem wurden sowohl im akuten Schub als auch in Remission Blutproben entnommen und archiviert. Mindestens 1x jährlich sollten die Patienten sich persönlich in der Universitätsmedizin vorstellen, optimaler Weise alle 3-4 Monate. Insgesamt sind 22 akute Schübe in 16 Patienten erfasst worden demgegenüber stehen 67 iTTP-Patienten in kontinuierlicher Remission.

Die Anzahl der Schübe pro Patient im Gesamtkollektiv (83 Patienten) betrug im Median 2 und die Erstdiagnose erfolgte mit 38 Jahren wohingegen das mediane Alter bei Studieneinschluss 49 Jahre war. Das Kollektiv war mit 61 Frauen auch in dieser Studie mehrheitlich weiblich (73%).

4. Differentialdiagnose der thrombotischen Mikroangiopathien bzw. Abgrenzung der TTP gegenüber anderen Erkrankungen und die Bedeutung von ADAMTS13 hierfür (**Originalarbeit V.**).

Da im Raum stand, dass COVID-19 zu einer thrombotischen Mikroangiopathie führen könnte, haben wir 85 Patienten mit COVID-19, die sich zwischen dem 3. März und dem 15. Mai 2020 in der Universitätsmedizin in Behandlung befanden, hierzu untersucht. Von 65 Patienten waren ausreichend klinisch und laborchemische Daten vorhanden, um eine Auswertung vorzunehmen. Diese waren im Schnitt 69 Jahre alt und mehrheitlich männlich (41 Männer gegenüber 24 Frauen). 19 Patienten hatten einen unkomplizierten, 29 einen komplizierten und 17 einen kritischen COVID-19 Krankheitsverlauf und 9 (14%) verstarben binnen des Studienzeitraums.

4.1.2. Ergebnisse und Diskussion bezüglich des Krankheitsverlaufs, der Charakteristik der akuten iTTP-Schübe, sowie zu den prognostischen Markern des akuten Schubes.

Die Diagnose der TTP wurde lange Zeit bei Patienten mit Petechien oder Blutungszeichen, Fieber, gleichzeitig auftretenden neurologischen Störungen in Kombination mit einer Thrombozytopenie, sowie einer hämolytische Anämie mit Fragmentozyten gestellt. Die sichere Differenzierung zu anderen TMAs war nahezu ausgeschlossen. So sind in den ersten großen klinischen Studien auch die Grenze zwischen HUS, TTP und anderen TTP-ähnliche Erkrankungen nicht eindeutig. Dies erklärt auch bei der ersten Symptombeschreibung der TTP die klassische Pentade mit neurologischen Symptomen, Fieber, Anämie, Thrombozytopenie und Niereninsuffizienz, die wir in unseren Kollektiven kaum noch sehen (**Originalarbeit I. und VI.**). Heute geht man bei der TTP eher von einer Trias aus neurologischen Symptomen, Anämie, und Thrombozytopenie aus, da eine Abgrenzung insbesondere

zum HUS, die Anzahl der vermeintlichen TTP Patienten mit Nierenbeteiligung vermindert.

Die bessere Abgrenzung der TMAs kam mit der Entdeckung (6) und Identifizierung der ADAMTS13 2001 (12) und der Möglichkeit ihre Aktivität zu bestimmen (42).

Während man sich mittlerweile einigen konnte, dass der ADAMTS13-Aktivitätsmangel grundlegend zur TTP Diagnose gehört, ist hingegen unklar, welche pathophysiologischen Mechanismen ineinandergreifen müssen, damit sich ein akuter TTP Schub entwickelt.

Alle Patienten in unseren Studienkollektiven weisen im akuten TTP Schub eine Thrombozytopenie und eine hämolytische Anämie (vermindertes Hämoglobin und deutlich erhöhte LDH, vermindertes Haptoglobin) mit Fragmentozyten auf (**Originalarbeit I., III. und VI.**).

Darüber hinaus haben die Patienten in der Hauptsache neurologische Symptome. Angefangen von Kopfschmerzen, Müdigkeit, Erschöpfung und Schwindel bis hin zu Seh-, Sprach- und Bewegungsstörungen, Parästhesien, Krampfanfällen und Koma (**Originalarbeit I., IV., VI.**).

In **Originalarbeit III.** haben wir einen Score zur Einteilung des Schweregrades des akuten TTP Schubes publiziert, der alle klinischen Symptome und Zeichen abdeckt, allerdings auch zeigt, dass es Patienten gibt, die nur laborchemische TTP Anzeichen aufweisen ohne klinisch auffällig zu werden. In unserer retrospektiven Studie waren dies 21 von insgesamt 219 Schübe über einen medianen Beobachtungszeitraum von 8,3 Jahren und 1 Schub von 21 in unserer prospektiven Beobachtungsstudie über gut 2 Jahre (**Originalarbeit III. und VI.**).

Insbesondere die Erstmanifestation der TTP ist mit 50% schweren und 32% moderaten Schüben verbunden (**Originalarbeit III.**). Dies ist in der Literatur (43), als auch im prospektiven Register (**Originalarbeit VI.**) bestätigt worden, in welchem zwei Erstdiagnosen mit schweren Verläufen auftraten, wohingegen alle anderen Schübe maximal moderat verliefen. Dies hat nichts mit der Erkrankung und ihrem Verlauf per se zu tun, sondern mit der Tatsache, dass viele Patienten bei der Erstdiagnose relativ spät in ein Klinikum eingewiesen werden und auch die Diagnose und die damit verbundene adäquate Therapie, nicht umgehend gestellt bzw. eingeleitet wird. Des Weiteren ist selbst in Deutschland eine therapeutische PEX nach Diagnosestellung nicht immer sofort und überall möglich. Nach erstmaliger Diagnose empfehlen wir den Patienten eine engmaschige Kontrolle, wodurch eine deutlich frühzeitigere

Behandlung, wenn nicht sogar eine prophylaktische Schubprävention, erfolgen kann. In unserer prospektiven Studie (**Originalarbeit VI.**) sehen wir, dass die Mehrheit von 52% der Patienten alle 3-4 Monate zur Kontrolle kommt und immerhin zusätzlich 34% einmal pro Jahr.
Nichts desto trotz verzeichnen wir auch vereinzelt noch sehr schwere bis hin zu letal verlaufende iTTP-Rezidiven viele Jahre nach Erstdiagnose (**Originalarbeit III**).

Insbesondere die ADAMTS13-Aktivität diskriminiert zwischen einer TTP und anderen Erkrankungen, die sich klinisch sehr ähnlich präsentieren. In unserer prospektiven Studie (**Originalarbeit VI.**) wurden anhand der ADAMTS13-Aktivität von 91 vermeintlichen TTP Patienten 6 wegen anderer Erkrankungen ausgeschlossen. Darunter waren ein Patient mit atypischem HUS, einer mit Evans Syndrom, und vier Patienten mit einem TTP-like Syndrom nach Lebertransplantation. Tatsächlich können Patienten nach Lebertransplantation ähnliche Zeichen wie TTP Patienten aufweisen. Auf der laborchemischen Seite zeigen sich niedrige Thrombozyten, erhöhtes LDH, sowie erniedrigte Hämoglobinwerte bei gleichzeitig auftretenden Fragmentozyten. Auf der klinischen Seite sind neurologische Auffälligkeiten zu erkennen. Nachdem die Leber der Hauptbildungsort der ADAMTS13 ist, ist eine TTP durchaus denkbar. In der Literatur finden sich hierzu auch einige Beschreibungen von Patienten, die nach Lebertransplantation eine TTP entwickeln (44-46). Unsere prospektive Untersuchung von Lebertransplantierten konnte allerdings keine klassischen TTP Ereignisse in diesem Kollektiv identifizieren. Eher konnten wir bestätigen, dass die für Leberpatienten typischen hohen Bilirubinwerte die klassischen ADAMTS13-FRETS-VWF73 Assays stören. Die Kombination aus einer durch die Lebertransplantation anfänglich verminderten ADAMTS13 Produktion und die zusätzlich falsch niedrige ADAMTS13-Aktivitätsmessung mittels FRETS-Assay durch hohe Bilirubinwerte, kann zu einer Falschdiagnose führen (Publikation in Arbeit).
Auch im Rahmen der COVID-19 Infektion rückten TMAs in den Blickpunkt. Grund hierfür war das Auftreten von thromboembolischen Ereignissen und der Nachweis von Mikrothomben in der Autopsie von an COVID-19 Verstorbenen, vor allem in der Mikrozirkulation der Lunge.
Darüber hinaus führen systemische Infektion und schwere Entzündung zu einer Endothelaktivierung oder Endothelschädigung mit massiver Freisetzung des VWFs aus den Endothelzellen. Daraus kann sich einerseits eine disseminierte intravaskuläre

Gerinnung (DIC) entwickeln, deren Erscheinungsbild ähnlich wie bei TMAs aussehen kann, andererseits kann sich ein Ungleichgewicht zwischen VWF und ADAMTS13 einstellen.

Ob das Ungleichgewicht zwischen VWF / ADAMTS13 in ähnlicher Weise zur Pathophysiologie bestimmter TMAs beiträgt wie bei der klassischen TTP bleibt unklar. Wir haben 65 an COVID-19 erkrankten Patienten auf Gerinnungsstörungen untersucht (**Originalarbeit V.**). In sieben ergab sich ein positiver DIC Score, berechnet aus D-Dimeren, INR, Fibrinogen und Thrombozytenzahl von ≥5, was auf eine DIC hindeutet. Während einige die Koagulopathie bei COVID-19 als TMA bezeichnen (47-49), konnten wir die Kennzeichen einer klassischen TMA in unseren 22 COVID-19 Patienten, die einer detaillierten Untersuchung auf TMA unterzogen wurden, nicht bestätigen. Allenfalls haben wir eine moderat verminderte ADAMTS13-Aktivität (18%-48% ADAMTS13-Aktivität) bei 4 der 22 Patienten gemessen. In der Literatur finden sich ebenfalls subnormale bis normale Werte für ADAMTS13, was eine TTP ausschließt. Auffällig waren die stark erhöhten Werte der VWF Aktivität und des VWF Antigens, sowie das erhöhte Verhältnis von VWF Antigen / ADAMTS13-Aktivität. Inwieweit dies pathophysiologisch relevant ist, bleibt fraglich, insbesondere da meist keine relevante Thrombozytopenie vorliegt. In nur 34% der COVID-19 Patienten waren Thrombozyten <150/nl gemessen worden, wobei in wiederum nur 3 Patienten eine schwere Thrombozytopenie (<50/nl) vorlag. Neben der Thrombozytopenie wäre eine mikroangiopathische hämolytische Anämie (MAHA) klassisch für eine TMA (50). Eine Anämie lag bei 52 der 65 Patienten vor und der Abfall der Hämoglobinwerte war kongruent zum Verlauf der COVID-19 Infektion. Die LDH Werte waren bei 95% der Patienten erhöht, aber aufgrund der normwertigen bzw. erhöhten Haptoglobinwerte wohl nicht Hämolyse-bedingt. Fragmentozyten waren auch bei nur zwei Patienten minimal erhöht, ansonsten normwertig. Aufgrund dieser Befunde wurde eine MAHA mit intravaskulärer Hämolyse in dieser Kohorte als unwahrscheinlich angesehen.

Während wir keinen Hinweis auf eine TTP bei COVID-19 Erkrankten finden konnten, und auch eine klassische TMA eher selten zu sein scheint, können wir umgekehrt COVID-19 als Trigger für einen akuten TTP Schub ausmachen (51).

Infektionen sind bereits seit längerem als potentielle Trigger eines akuten TTP Schubes in der Diskussion (31). In **Originalarbeit I.** haben wir beschrieben, dass bei 53 von 103 akuten Schüben ein potentieller Auslöser vorangegangen war. Dabei handelte es sich in 57% der Fälle um Infektionen und hierbei wiederum insbesondere

um gastrointestinale Infektionen. Mit 17% steht an zweiter Stelle die Schwangerschaft als Schubauslöser eines akuten TTP Schubes im Verdacht (**Originalarbeit I.**). Dies deckt sich mit zahlreichen Angaben aus der Literatur (32, 52).
In unserem prospektiven Register (**Originalarbeit VI.**) haben wir ebenfalls eine Schwangerschaft bei einer iTTP Patientin verfolgt, in der sich ein Schub entwickelte. Speziell aber hereditäre TTP Patientinnen sind besonders betroffen, da es in diesem Fall vor allem häufig der Auslöser des initialen TTP Schubes im jungen Erwachsenenalter zu sein scheint (53) (54, 55).

Der schwere ADAMTS13-Aktivitätsmangel (<10%) gilt mittlerweile als Nachweis für das Vorliegen einer TTP. Im akuten Schub ist bei allen Patienten eine schwere Defizienz nachweisbar (Abb.5 jeweils oben, Abb. 6 rechts), die sich während der Akutbehandlung bis hin zur Remission bei iTTP-Patienten meistens erholt (Abb. 6 rechts) (**Originalarbeiten I., III. und VI.**). Allerdings gibt es auch Patienten, die konstant niedrige ADAMTS13-Aktivitäten auch nach einem akuten Schub beibehalten (Abb. 5 link und Abb. 6).

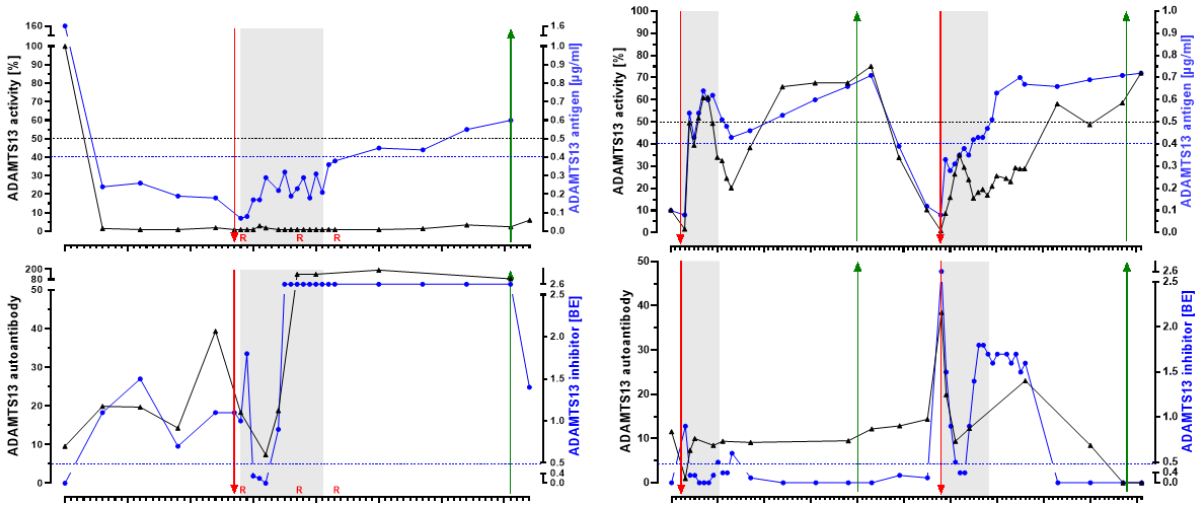


Abb. 5: Verlauf TTP-spezifischer Marker vor, während und nach einem akuten iTTP-Schub. Rechts: ADAMTS13-Aktivität, Antigen, Autoantikörper und Hemmkörper eines Patienten mit einem akuten Schub. Links: ADAMTS13-Aktivität, Antigen, Autoantikörper und Hemmkörper eines Patienten mit zwei akuten Schüben. Der rote Pfeil kennzeichnet den Schubbeginn, der grüne Pfeil den Beginn der Remission. Grau unterlegt ist der Zeitraum der PEX und mit R sind die Rituximab Gaben markiert.

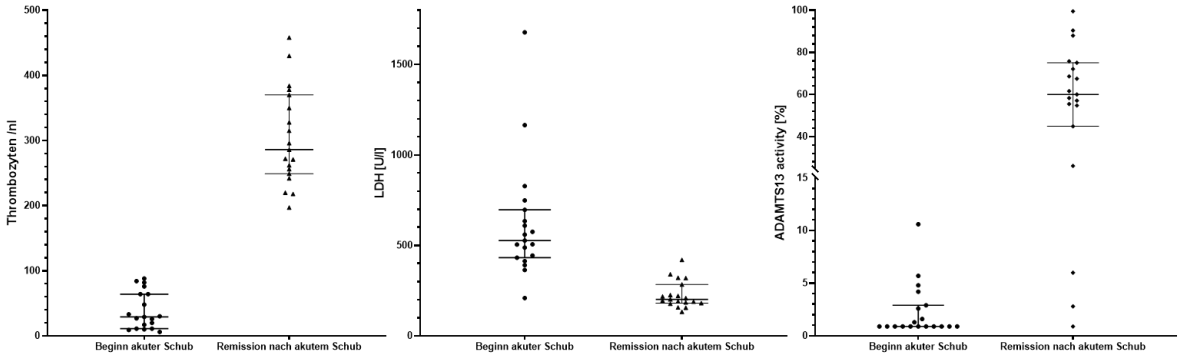


Abb. 6: Thrombozyten (links), LDH Werte (Mitte) und ADAMTS13-Aktivität (rechts) von 15 Patienten mit 20 akuten iTTP-Schüben zu Beginn des akuten Schubes vor PEX und zu Remissionsbeginn 30 Tage nach der letzten PEX (**Originalarbeit VI.**).

Nach Rückgang der klinischen Symptome und Normalisierung der Laborwerte (Abb. 6) galten TTP Patienten lange als geheilt. Allerdings sind erneute Schübe häufig und diese sind immer potentiell lebensbedrohlich. Daher haben wir in einer retrospektiven Betrachtung unseres iTTP Kollektivs ein besonderes Augenmerk auf die Rückfallrate und potentielle Einflussfaktoren gelegt.

Mit der Einführung von Rituximab steht seit 1997 ein monoklonaler anti-CD-20 Antikörper zur Verfügung, der eine B-Zelldepletion bewirkt. In der Universitätsmedizin Mainz wurde Rituximab seit 2003 bei iTTP-Patienten eingesetzt, da man sich eine Reduktion der ADAMTS13-Autoantikörperproduktion bei der iTTP erhoffte. Inwieweit sich die Gabe von Rituximab positiv auf die Behandlung des akuten iTTP-Schubes und potentielle Rückfälle auswirkt, war nicht bekannt. Es gibt hierbei mehrere Ansätze für den Einsatz von Rituximab. Bei therapierefraktären Patienten, sowie beim Beginn von akuten iTTP Episoden oder präventiv bei Patienten in Remission mit anhaltender oder erneut auftretender ADAMTS13 Defizienz (33, 56-58).

In unserer retrospektiven Untersuchung, die 2003 mit dem ersten Einsatz von Rituximab in der Universitätsmedizin Mainz startet, wurden bis 2014 70 iTTP-Patienten eingeschlossen (**Originalarbeit III.**).

Von 219 gut dokumentierten Schüben wurden 69 akute Schübe (37 Patienten) unter anderem mit Rituximab und 150 akute Schübe (33 Patienten) mit der Standardtherapie ohne Rituximab behandelt. Der Einsatz von Rituximab erfolgte damals ausschließlich

bei refraktären akuten iTTP-Schüben oder bei Patienten mit frühen Rezidiven. Unabhängig von der Rituximab-Gabe hatten 59% des Kollektives nach ihrem ersten akuten Schub mindestens einen weiteren akuten Schub. Dies liegt etwas höher als die in der Literatur angegebenen 20% bis 50% (41, 59). Bei der Auswertung der Rückfallrate in Bezug zur Rituximab Gabe konnten wir keinen signifikanten Unterschied ausmachen. Die Rückfallrate bei Patienten mit Rituximab betrug 2,3% pro Monat, bei denen ohne Rituximab-Gabe 2,6% (Anderson-Gill Modell p= 0,729).

In einer zweiten Auswertung haben wir die Rezidiv-freie Zeit von 45 Patienten mit Initialdiagnose nach 2003 (also nach Einführung von Rituximab in die TTP Behandlung) untersucht. Eine Kaplan-Meier Analyse zur Untersuchung des rückfallfreien Überlebens wies keinen Unterschied zwischen den beiden Patientengruppen auf (17 mit Rituximab, 28 ohne Rituximab Therapie; p-Wert= 0,131).

Allerdings lag die Rückfallquote bei Patienten mit Rituximab-Gabe im Initialschub bei 29% und bei denen ohne Rituximab bei 50%. Ein positiver, wenn auch nicht signifikanter, Trend ist ebenfalls beim ereignisfreien Überleben zu erkennen. 94% der Patienten, die mit Rituximab behandelt wurden, hatten nach einem Jahr noch kein Rezidiv erlitten und 79% auch nach 1000 Tagen noch nicht. Patienten ohne Rituximab waren nach 1 Jahr zu 82% bzw. nach 1000 Tagen zu 57% Rezidiv-frei.

In der Literatur finden sich hierzu sehr unterschiedliche Zahlen. So sind 10% bis 29% Rückfallraten mit Rituximab-Behandlung gegenüber 43% bis 57% ohne Rituximab Gabe publiziert (33, 56, 60-62).

Wir konstatieren, dass Rituximab die Häufigkeit von Rückfällen verringern kann; allerdings können wir nur eine Tendenz und keinen signifikanten Effekt für unsere Kohorte angeben. Des Weiteren scheint der Einfluss vor allem im ersten Jahr nach der akuten Episode am größten zu sein. Unsere Daten ähneln denen von Froissart et al., die bei ihren refraktären Patienten, die mit Rituximab behandelt wurden, keinen signifikanten Unterschied in der Rückfallrate feststellen konnten (63).

Was wir allerdings sehen, ist, dass bei Patienten, die direkt beim Auftreten eines „laborchemischen“ Schubes und bevor sich klinische Symptome einstellten, Rituximab erhielten, die Anzahl der PEX deutlich reduziert ist. Die frühzeitige Verabreichung von Rituximab führt zu einer schnelleren Remission und einer geringeren Anzahl von notwendigen PEX-Sitzungen (22). Dies führt zur Überlegung, ob bei all jenen Patienten

mit persistierendem oder wiederauftretendem schweren ADAMTS13 Mangel Rituximab verabreicht werden sollte und dies noch bevor ein Thrombozytenabfall oder ein LDH-Anstieg erkennbar ist. Hie et al. zufolge führt eine präventive Rituximab-Behandlung bei asymptomatischen Überlebenden von mindestens einem iTTP-Schub zur Reduktion von Rückfällen im Vergleich zu Patienten, die kein Rituximab erhalten (58). Im Gegensatz dazu wurde die präventive Behandlung, wie sie Owattanapanich et al. (33) und Jestin et al. (56) empfehlen, von anderen Autoren jedoch in Frage gestellt (64).

Nach heutigen Stand, über 5 Jahre nach Erscheinen der **Originalarbeit III.**, ist Rituximab in die Standardtherapie der iTTP eingegangen (35).

Wie bei vielen Autoimmunerkrankungen, sind auch bei der iTTP Frauen häufiger betroffen als Männer (65-67). Sowohl in den retrospektiven wie der prospektiven Kohortenstudie waren Frauen mit einem Anteil von 77%, 76% bzw. 73% vertreten (**Originalarbeit I., III. und VI.**). Allerdings scheinen Männer, die an einer iTTP erkranken, unabhängig von der Behandlung mit Rituximab, deutlich schwerer betroffen zu sein (**Originalarbeit III.**).

Die Rezidivrate, definiert als akuter Rückfall pro 100 Patientenmonate, beträgt für das Gesamtkollektiv 2,6% pro Monat. Bei der Betrachtung der Rückfallrate nach Geschlecht zeigt sich, dass Frauen eine Rückfallrate von 2,4% pro Monat haben, Männer hingegen von 3,5% pro Monat. Folglich haben Männer ein 1,5fach höheres Risiko eines erneuten akuten Schubes ($p= 0,009$) (**Originalarbeit III.**). Zudem verlaufen die akuten Schübe von Männern schwerer als bei Frauen. Das Phänomen des schwereren Krankheitsverlaufes ist auch bei anderen Autoimmunerkrankungen wie der Multiplen Sklerose oder dem systemischem Lupus Erythematodes beschrieben (66, 68) und Fakhouri et al. haben für männliche iTTP-Patienten ebenfalls eine signifikant höhere Rückfallneigung dargestellt (69).

In unseren retrospektiven wie prospektiven Registern haben iTTP-Patienten im Median 2 Schübe über den jeweiligen Beobachtungszeitraum (**Originalarbeiten III. und VI.**), wobei die Bandbreite von 1 Schub bis hin zu 23 Schüben reicht.

Nachdem mit jedem Schub ein massives Mortalitäts- und Morbiditätsrisiko einhergeht, steht neben der Behandlung des Schubes, insbesondere dessen frühzeitiges Erkennen und, wenn möglich, sogar dessen Vermeiden im Vordergrund. Daher haben

wir in einer prospektiven Beobachtungsstudie versucht potentielle prädiktive Marker eines TTP Rezidivs zu identifizieren (**Originalarbeit VI.**).

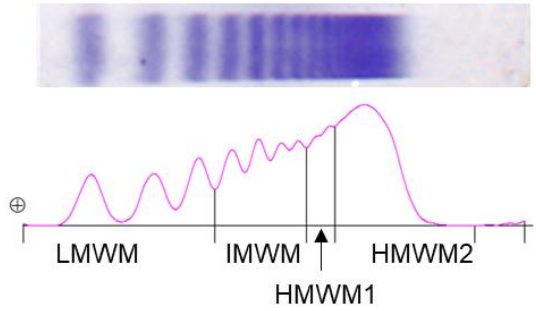
Im Studienzeitraum von Juli 2016 bis August 2018 konnten wir 83 iTTP-Patienten, im akuten Schub oder in Remission befindlich, rekrutieren und prospektiv weiterverfolgen. Nachdem eine anhaltende ADAMTS13 Defizienz, aber auch der Abfall der ADAMTS13-Aktivität als Risikofaktor für das Wiederauftreten eines akuten Schubes gelten (59, 70), wurde die ADAMTS13-Aktivität, das ADAMTS13 Antigen und der ADAMTS13 Hemmkörper über den Studienzeitraum verfolgt. Auch wenn die ADAMTS13-Aktivität ein guter Indikator ist, reicht sie bei weitem nicht aus, um einen Rückfall vorherzusagen (71, 72). In unserem Kollektiv sehen wir, dass 13 der 83 Patienten bis zu zwei Jahre lang in klinischer Remission blieben, obwohl ihre ADAMTS13-Aktivität <10% des Normalwertes betrug. Ein weiterer potenzieller Frühmarker für ein drohendes iTTP-Rezidiv scheint der ADAMTS13 Konformationsindex zu sein (73). Die Bindung von Autoantikörper an ADAMTS13, was spezifisch für die iTTP ist, bewirkt, dass die Metalloprotease ADAMTS13 in offener Konformation (entspricht einem Konformationsindex >0,5) vorliegt (74). Tatsächlich wiesen in unserer Kohorte 13 von 14 Patienten zwei Tage bis 28 Wochen vor einem akuten Rezidiv eine offene Konformation auf, unabhängig von der ADAMTS13-Aktivität. Allerdings sehen wir auch bei den Patienten, die über den Studienzeitraum in Remission blieben, dass alle 13 Patienten mit ADAMTS13 <10% und die Hälfte derjenigen mit ADAMTS13 ≥10%, ebenfalls eine offene ADAMTS13-Konformation aufwiesen (**Originalarbeit VI.**).

Der VWF ist das bisher einzige bekannte Substrat der ADAMTS13. Im Tiermodell mit ADAMTS13- und VWF-Doppelknockout-Mäusen ($ADAMTS13^{-/-}$ $VWF^{-/-}$) wiesen Chauhan et al. (75) nach, dass ein VWF-Mangel den prothrombotischen Zustand eines ADAMTS13-Mangels aufhebt. Dies deutet darauf hin, dass der hyperadhäsive, hochmolekulare VWF der unverzichtbare Mediator für die Thrombozytenadhäsion und -aggregation ist. Von dieser Tatsache ausgehend, haben wir uns der Untersuchung der VWF Multimerverteilung im Plasma der iTTP-Patienten gewidmet. Die Untersuchung von ultralangen VWF Multimeren wird seit gut 3 Jahrzehnten bei der TTP eingesetzt (5). Während die herkömmliche VWF Multimeranalyse allerdings offenkundig schwierig durchzuführen und zu standardisieren ist (76), ermöglicht der kommerziell erhältliche Hydragel 11 Von Willebrand Multimerkit eine reproduzierbare

Bestimmung der VWF Multimerverteilung (77-79). Allerdings sind bei dieser Methode, die für die Diskriminierung des VWS Typ 1 von Typ 2A und 2B etabliert ist, keine Triplettstrukturen des VWFs erkennbar und die ultralangen VWF Multimere nicht einfach individuell abgrenzbar (Abb. 3 und Abb. 7). In einem ersten Schritt haben wir Proben von TTP Patienten und Kontrollen mit konstanten VWF Mengen auf die Gele aufgetragen. Nach der elektrophoretischen Trennung war eine Quantifizierung von nieder- (LMWM), mittel- (IMWM) und hochmolekularen (HMWM) VWF Multimeren möglich, wobei die hochmolekularen VWF Multimere in eine HMWM1- und eine HMWM2-Fraktion aufgeteilt wurden. Mittels der Densitometrie konnte reproduzierbar das Verhältnis von HMWM2 / LMWM ermittelt werden. Mit dieser VWF Multimer Ratio (VWF MM Ratio) sind Unterschiede zwischen normalen und iTTP Plasmen, sowie zwischen verschiedenen Krankheitszuständen bei iTTP-Patienten (Abb. 7) abzubilden. Ein Referenzbereich von 1,1 bis 3,5 wurde für die VWF MM Ratio ermittelt (Originalarbeit VI).

Patienten in Remission haben eine signifikant höhere VWF Multimer Ratio, wenn ihre ADAMTS13-Aktivität <10% liegt gegenüber den Patienten in Remission, die eine ADAMTS13-Aktivität von ≥10% aufweisen (**Originalarbeit VI.**).

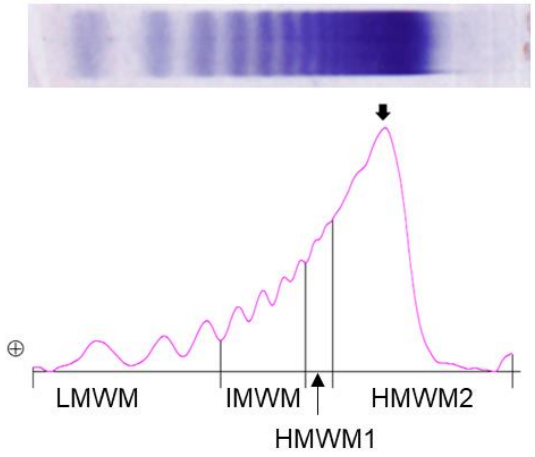
Beispielhafte SDS-Gelelektrophoresen:



eines gesunden Probanden

VWF Multimer Ratio

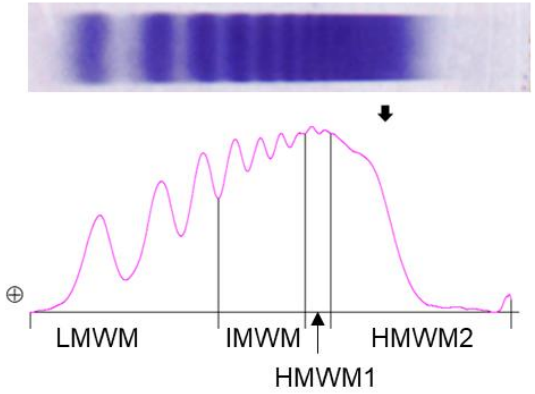
des gesunden Kontrollkollektivs: 1,2 – 3,4



eines Patienten mit iTTP

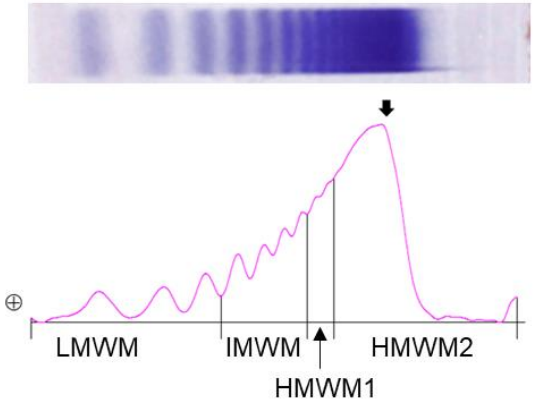
VWF Multimer Ratio

vor dem akuten Schub: 4,4



VWF Multimer Ratio

zu Beginn des akuten Schubes: 0,8



VWF Multimer Ratio

nach dem akuten Schub in Remission: 3,9

Abb. 7: VWF Multimerverteilung nach elektrophoretischer Auftrennung und densitometrischer Auswertung bei einem gesunden Probanden, sowie bei einem iTTP-Patienten vor, zu Beginn und nach einem akuten Schub.

Zu Beginn eines akuten Schubes konnten wir, bereits vor dem ersten therapeutischen PEX, ein Absinken der VWF MM Ratio auf (sub)normale Werte sehen, die am ehesten durch den Verbrauch der überwiegend hochmolekularen VWF Multimeren in den Mikrothromben erklärbar ist (5). Überraschend war, dass wir einige Tage bis Wochen vor einem akuten iTTP-Rezidiv eine signifikant höhere VWF MM Ratio nachweisen konnten, als bei den Patienten, die sich in Remission über den ganzen Studienzeitraum befanden. Dabei waren die ADAMTS13-Aktivitäten bei 5 (von 14) Patienten vor dem akuten Schub >10%. In der Literatur ist beschrieben, das IL-6 die Spaltung des VWFs durch ADAMTS13 unter Flussbedingungen hemmte (80) und IL-8 und TNF-α die Freisetzung von VWF Multimeren mit hohem Molekulargewicht erhöhten (81). Zudem sind signifikant erhöhte Plasmazytokinspiegel bei akuten iTTP Anfällen gezeigt (82). In unserem Kollektiv ergab die Messung von IL-6 und CRP keinerlei erhöhte Werte vor einem akuten Schub, weswegen die sehr hohe VWF MM Ratio der Patienten vor einem Schub, trotz messbarer ADAMTS13-Aktivität, ungeklärt bleibt. Dennoch deuten unsere Daten darauf hin, dass die proteolytische Spaltung des VWFs bei einigen iTTP-Patienten, auch bei messbarer ADAMTS13-Aktivität, beeinträchtigt sein kann und dass bei diesen Patienten ein hohes Risiko für einen klinischen iTTP Rückfall besteht.

Unsere Studie ist relativ klein, die Zahl der Patienten, die einen klinischen Rückfall entwickeln, ist begrenzt und die Nachbeobachtungszeit war relativ kurz. Dennoch lässt sich zusammenfassend sagen, dass zwar bisher kein Marker allein ausreicht, um einen akuten Rückfall vorherzusagen, jedoch eine Kombination vielleicht schon. Eine schwerer ADAMTS13 Defizienz, eine offene ADAMTS13 Konformation und eine hohe VWF MM Ratio könnten zusammen ein zuverlässiger Indikator für ein drohendes Rezidiv sein und damit ein Grund, eine prophylaktische Therapie in Betracht zu ziehen.

4.2. Langzeitfolgen der TTP

4.2.1. Zugrundeliegende Patientenkollektive

Dem folgenden Abschnitt liegen eine Voruntersuchung und zwei Teilbereiche einer langjährigen prospektiven Patientenbeobachtung zugrunde (Originalarbeiten I., II. und IV.)

1. Die retrospektive Voruntersuchung zeigt, dass iTTP-Patienten nach einem akuten Schub mit Langzeitfolgen kämpfen (**Originalarbeit I.**).

(siehe 4.1.1.)

2. Prospektive Kohortenstudie zur Untersuchung von kognitiven wie psychischen Langzeitfolgen nach einem akuten iTTP-Schub und potentielle Zusammenhänge zur Schwere des bzw. der akuten iTTP-Schubes/Schübe (**Originalarbeit II.**).

Von Oktober 2012 an wurden alle iTTP-Patienten, die in der Universitätsmedizin Mainz behandelt wurden, konsekutiv in einem iTTP Register aufgenommen. Im Juni 2013 wurden die bis dahin bekannten iTTP-Patienten eingeladen an einer Fragebogenstudie zu Langzeitfolgen der iTTP teilzunehmen. Patienten über 18 Jahre, die mindestens einen akuten iTTP-Schub erlitten hatten und sich zum Zeitpunkt der Befragung in Remission befanden, erhielten einen standardisierten Fragebogen. Die Befragung fand ein Jahr später, im Juli 2014, erneut statt. Patienten, die im Zeitraum von 2013 bis 2014 erstdiagnostiziert wurden, haben den Fragebogen ebenfalls erhalten und wurden zur ersten Fragebogenrunde gezählt. Neben den iTTP-Patienten wurde ein Kontrollkollektiv aus 52 gesunden Probanden befragt. Der Frauenanteil lag hier bei 61% und die Probanden waren durchschnittlich 35 Jahre.

Von 149 iTTP-Patienten in der ersten Befragungsrunde und 127 in der zweiten Befragungsrunde haben wir auswertbare Rückmeldungen von 85 respektive 71 Patienten (Abb. 8). Insgesamt haben 104 iTTP-Patienten bei beiden Befragungen, 2013 und 2014, teilgenommen, wovon bei 60 eine komplett auswertbare Krankenakte vorlag. Bei beiden Befragungsrunden war der Anteil an Frauen mit 80% resp. 85% passend zur Geschlechtsverteilung innerhalb der

Patientenkohorte selbst. Das Alter der Studienteilnehmer lag bei 46 bzw. 45 Jahren.

3. Prospektive Studie mit dem Fokus auf Resilienz und Lebensqualität von iTTP-Patienten (**Originalarbeit IV.**).

147 und 142 iTTP-Patienten erhielten 2015 und 2016 erneut einen Fragebogen, der etwas abgeändert wurde im Vergleich zu den ersten beiden Befragungsrunden. Die Rücklaufquoten waren annähernd identisch zu den ersten beiden Jahren (Abb. 8). Bei den Befragungen, 2015 und 2016, waren Ergebnisse von insgesamt 104 individuellen Patienten auswertbar. Das durchschnittliche Alter lag bei 48 bzw. 51 Jahren und der Frauenanteil war erneut bei 76% bzw. 82%. Ein Kontrollkollektiv wurde erstellt, indem 300 alters- und geschlechts-gematchte Personen aus der Bevölkerung aus Mainz und Umgebung angeschrieben wurden. 134 auswertbare Rückmeldungen haben wir erhalten.

Über alle vier Befragungen hinweg haben 38 Personen an jeder Befragung teilgenommen und insgesamt 133 verschiedene iTTP-Patienten (Abb. 8).

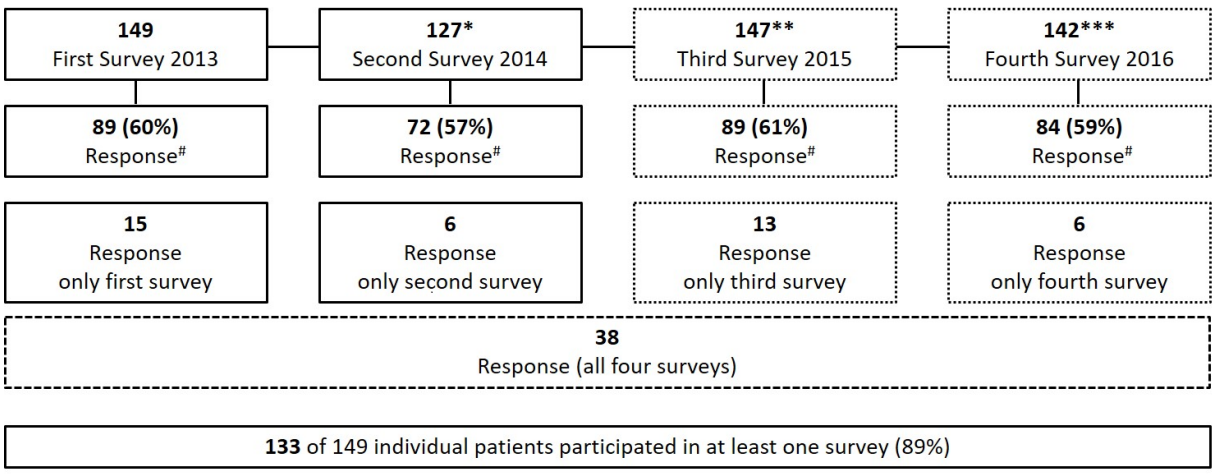


Abb. 8: Rekrutierung und Antwortrate der iTTP-Patientenkohorte in allen vier Befragungsrunden.

*Zwischen der ersten und zweiten Erhebung starben zwei Teilnehmer. 19 Patienten mit Initialschub nach 2013 wurden 2014 noch nicht wieder befragt. **Ein neuer Patient. ***Fünf Patient waren unbekannt verzogen. #Nicht jeder Fragebogen auswertbar.

4.2.2. Ergebnisse und Diskussion bezüglich der Langzeitfolgen der iTTP

Das Überleben des akuten Schubes, der nach wie vor eine Mortalität von 10-20%* (vor Caplacizumab-Einführung) aufweist, ist selbstverständlich das primäre Ziel bei der Behandlung der iTTP. Jedoch zeigt sich darüber hinaus, dass die iTTP-Patienten nachhaltig Einschränkungen erleben und unter Langzeitfolgen leiden. Aus der ersten retrospektiven Patientenbetrachtung, von 21 Patienten mit 104 akuten Schüben, ist hervorgegangen, dass 11 Patienten wegen Depressionen sich nach der Erstdiagnose in Behandlung begeben mussten (**Originalarbeit I.**). Zudem wurden sehr viele Einträge in den Patientenakten hinsichtlich Ängsten, eingeschränkter Lebensqualität und einer deutlich verminderten Leistungsfähigkeit vermerkt, die der Patient im Arztgespräch geäußert hat. Darüber hinaus litten sechs Patienten in Folge ihrer Schub-bedingten Schlaganfälle an Epilepsie, vier Patienten entwickelten eine Niereninsuffizienz (**Originalarbeit I.**).

Um Langzeitfolgen systematisch zu untersuchen und darauf adäquat reagieren zu können, haben wir die iTTP-Patienten, die an der Universitätsmedizin behandelt oder konsiliarisch gesehen wurden, prospektiv über 4 Jahre, von 2013 bis 2016, untersucht (Abb. 8).

Ziel der ersten beiden Befragungen war es die Prävalenz von Depressionen und kognitiven Defiziten bei Patienten zu untersuchen, die einen akuten iTTP-Schub überlebt hatten.

Hierfür erhielten die Patienten einen standardisierten Fragebogen, der zwei validierte Fragebögen beinhaltete, den IDS-SR für die Erfassung depressiver Symptome und den FLei, um die geistige Leistungsfähigkeit zu überprüfen.

In der zweiten Befragungsrunde lag die Gewichtung mehr darauf, zu sehen in wieweit Lebensumstände, sowie Persönlichkeit und Widerstandsfähigkeit der Patienten einen Einfluss auf Depressionen, Ängste und Lebensqualität haben. Mit den beiden weltweit gängigen Fragebögen zu Depressionen (PHQ-9) und Ängsten (GAD-7) wurde die Patientenkohorte befragt. Darüber hinaus ist mit dem FLei erneut die geistige Leistungsfähigkeit, mit dem RS-11 die Resilienz, mit dem LOT-R die Lebenseinstellung und dem QLQ-C30 die Lebensqualität erfasst worden.

In allen vier Untersuchungen ergab sich, dass iTTP-Patienten deutlich verstärkt unter Depressionen leiden. In der Befragung 2013 waren es 72,6%, 2014 59,2% (**Originalarbeit II.**) und in 2015 61,4% und 2016 63,0% (**Originalarbeit IV.**) der iTTP-Patienten, die depressive Symptome aufwiesen.

Tab. 1: Ergebnisse der iTTP-Patienten und der Kontrollgruppen hinsichtlich depressiver Symptome von 2013 bis 2016, ermittelt mit Hilfe standardisierter Fragebögen (IDS-SR und PHQ-9).

	IDS-SR 2013	IDS-SR 2014	IDS-SR Kontrollen	PHQ-9 2015	PHQ-9 2016	PHQ-9 Kontrollen
Anzahl auswertbarer Ergebnisse	84	71	52	88	81	133
Median	20	18	7	5	7	3
IQR	12-34	9-33	4-9	2-10	2,5-12,5	1-6
Bereich	0-59	0-61	0-25	0-23	0-23	0-18
Depressive Symptome						
keine	27,4%	40,8%	86,5%	38,6%	37,0%	66,2%
milde	32,1%	21,1%	13,5%	35,2%	28,4%	29,3%
moderate	26,2%	26,8%	0,0%	14,8%	17,3%	3,8%
schwere bzw. moderate bis schwere	7,1%	7,0%	0,0%	10,2%	16,0%	0,8%
sehr schwere bzw. schwere	7,1%	4,3%	0,0%	1,1%	1,2%	0,0%

Im IDS-SR wird unterschieden in keine, milde, moderate, schwere und sehr schwere depressive Symptome. Keine Anzeichen für eine Depression zeigten 27,4% (2013) bzw. 29,0% (2014) der iTTP-Patienten, wohingegen es im Kontrollkollektiv 86,5% waren (Tab. 1). Von einer klinisch relevanten Depression wird ausgegangen, wenn eine IDS-SR Score von >25 vorliegt. Das entspricht der Einteilung von moderaten und schweren depressiven Symptomen. 40,4% (2013) bzw. 38,1% (2014) der iTTP-Patienten wiesen folglich eine klinisch relevante Depression auf, im Kontrollkollektiv hingegen niemand (Tab. 1). Im Median liegen die iTTP-Patienten allerdings unterhalb dieser Grenze mit 20 (IQR 12-34) und 18 (IQR 9-33), jedoch signifikant ($p < 0,001$) höher als das Kontrollkollektiv mit 7 (IQR 4-9) (**Originalarbeit II.**). In der 2015 und 2016 durchgeführten Befragung zeigt sich mit dem verwendeten PHQ-9 ein ähnliches Bild. Unterschieden wird hier in keine, milde, moderate, moderat bis schwere und schwere depressive Symptome. Keine depressiven Anzeichen sind bei 38,6% (2015) bzw. 37,0% (2016) zu verzeichnen, und im Kontrollkollektiv bei 66,2% (Tab. 1). Von

einer Major Depression wird im PHQ-9 ausgegangen, wenn ein Punktescore von ≥10 vorliegt. Dies ist in 2015 bei 26,1% und in 2016 bei 34,5% der iTTP-Patienten gegeben (**Originalarbeit IV.**). Dies ist vergleichbar mit den Ergebnissen von Deford et al., welche in ihrem iTTP-Patientenkollektiv 19% Major Depressionen angeben (83). Im Kontrollkollektiv lag der Anteil an Major Depressionen bei 4,6%. Im Median liegen die iTTP-Patienten hier ebenfalls unterhalb dieser Grenze mit einmal 5 (IQR 2-10) und einmal 7 (IQR 2,5-12,5), aber wiederum auch signifikant ($p < 0{,}001$ und $p < 0{,}0001$) höher als das Kontrollkollektiv mit 3 (IQR 1-6). Damit sind bei beiden Fragebögen (IDS-SR und PHQ-9) vergleichbare Ergebnisse für die iTTP-Patientenkohorte erzielt worden. Der Anteil der iTTP-Patienten mit Depression übersteigt deutlich die 12-Monats Prävalenz für Depressionen in der Deutschen Bevölkerung. Diese ist mit 9,3% angegeben, wobei Frauen mit 12,4% eine höhere Prävalenz aufweisen als Männer mit 6,1% (84). Unser mittels IDS-SR befragtes Kontrollkollektiv wies 2014 mit 13,5% eine der deutschen Bevölkerung vergleichbare Prävalenz für Depressionen auf. Das mittels PHQ-9 befragte Kontrollkollektiv 2016 lag mit 33,9% deutlich höher, allerdings waren nur 4,6% mit einer Major Depression eingestuft. Unsere Ergebnisse sind analog mit Untersuchungen von Han et al., die in ihrem TTP-Patientenkollektiv eine Punktprävalenz der klinisch relevanten Depression von 46% angeben (85). Han et al. verwendeten den Beck Depression Inventory, den Test mit dem der IDS-SR gegengetestet wurde (85). Daher kann man davon ausgehen, dass die Ergebnisse sehr gut vergleichbar sind. Ebenfalls mit dem Beck Depression Inventory hat die Arbeitsgruppe von Chaturvedi et al. gearbeitet. Sie berichten von einem Anteil von 80,8% der iTTP-Patienten mit mindestens milden depressiven Symptomen (86). Sie geben an, dass zum Befragungszeitpunkt nur bei 47,9% der Patienten eine Depression diagnostiziert worden war. In unseren Untersuchungen waren es mit 20% der Patienten, die sich zum Zeitpunkt der Befragung in Psychotherapie befanden, deutlich weniger (**Originalarbeit II.**).

Darüber hinaus haben wir untersucht, inwieweit die Schwere des Krankheitsverlaufes eine Rolle bei der Entwicklung einer Depression und deren Ausprägung haben könnte. Bei 60 (von 104) iTTP-Patienten lagen die komplette Krankengeschichte vor, sodass wir die Anzahl der Schübe und das Auftreten von neurologischen Symptomen während der akuten Schübe mit dem Schweregrad der depressiven Symptomatik im IDS-SR korrelieren konnten (**Originalarbeit II.**). Eine Assoziation zwischen der Anzahl der Schübe und der Schwere der Depression war nicht auszumachen ($r_s = 0{,}157$) ebenso

wenig spielte der Umstand, neurologische Symptome erlitten zu haben, eine signifikante Rolle (p =0,781). Einen Unterschied machte es allerdings, ob die Patienten bisher nur einen Schub erlitten hatten oder 2 und mehr Schübe (p =0,011). Auch der Abstand zum letzten Schub spielte möglicherweise eine Rolle. Patienten mit einem Schub binnen des letzten Jahres (IDS-SR Score Median 26, IQR 19-45) haben tendenziell einen höheren IDS-SR Score als Patienten, deren Schub mehr als ein Jahr (Median 20, IQR 10-33) zurückliegt (p =0,063, NS) (**Originalarbeit II.**). Bei der Betrachtung der IDS-SR Ergebnisse (n=55) 2013 zu 2014 sehen wir, dass binnen dieses Jahres die Patienten mit keinen und die mit schweren Depressionen kaum Veränderungen zeigen, wohingegen die Varianz in den milden und moderaten Depressionsklassen am größten ist (**Originalarbeit II.**). Holmes et al. bestätigten einige Zeit später unsere Erkenntnisse (87). Sie haben ebenfalls keine Korrelation zwischen dem Schweregrad und der Anzahl der Schübe mit dem Ausmaß der psychischen Beeinträchtigung detektieren können (87).

Bei schwerwiegenden Erkrankungen spielen neben den Depressionen auch Ängste eine entscheidende Rolle. Im Jahr 2015 wiesen 24,1% leichte, 13,8% mittlere und 5,7% schwere Angstsymptome auf (**Originalarbeit IV.**). Ein ähnliches Bild ergab sich 2016, hier hatten 36,3% der Patienten leichte, 13,8% mittelschwere und 3,8% schwere Angstzustände. Interessanterweise zeigt sich aber insgesamt nur ca. bei der Hälfte der Patienten (2015 43,7% und 2016 53,7%) eine Angstsymptomatik wohingegen ca. 2/3 depressive Symptome aufwiesen. Nichtsdestotrotz war die Prävalenz von Angststörungen in der gesamten iTTP-Kohorte in beiden Erhebungen signifikant höher (2015 p <0,035; 2016 p <0,008) als in der Kontrollgruppe (**Originalarbeit IV.**). Insbesondere war der Anteil klinisch relevanter Angststörungen in der iTTP-Kohorte 2015 (19,5%) und 2016 (17,6%) deutlich höher als in der Kontrollgruppe (8,4%). Die Arbeitsgruppe von Peyvandi gibt ebenfalls Angststörungen mit 20% in ihrem Kollektiv an und bestätigt unsere Erkenntnisse, sowie den Umstand, dass diese weniger auftreten als Depressionen (43%) (88).

Grundsätzlich kommt es bei vielen Erkrankungen zu einem gehäuften Auftreten von Depressionen und Angststörungen. Dies ist sowohl bei akuten Erkrankungen wie Myokardinfarkt (89) oder dem Schlaganfall (90-92) als auch bei chronischen Erkrankungen wie der Multiplen Sklerose (93) oder dem systemischen Lupus Erythematodes (94) beschrieben.

Mit Depressionen und Angststörungen gehen auch ein vermindertes Wohlbefinden ebenso wie eine geringere Lebensqualität einher. Die Widerstandsfähigkeit und Lebenseinstellung sind wiederum wichtige Einflussgrößen bei der Entstehung von Depressionen und Ängsten, der Verarbeitung von schwierigen Situationen und sind somit unmittelbar an Lebensqualität und Wohlbefinden beteiligt.

In der zweiten Befragungsrunde wurde daher das Augenmerk auf genau diese Faktoren gelegt. Mit drei Fragebögen wurde die Resilienz, die Lebenseinstellung, sowie die Lebensqualität erfragt und wiederum mit Depressionen korreliert. Darüber hinaus wurden Einflussfaktoren bezüglich der Entwicklung von Depressionen, wie die Lebenssituation (Partnerschaft und Kinder), der körperliche Zustand (sportliche Fitness und zusätzliche Erkrankungen) sowie das Geschlecht (95) und das Alter erfasst (96) (**Originalarbeit IV.**).

Die Resilienz der iTTP-Patienten war im Vergleich zur gesunden Kontrollkohorte signifikant schlechter (2015 $p<0,04$; 2016 $p<0,0001$). Im Jahr 2015 wiesen die Patienten einen Resilienz Score von Median 60 (IQR 49,5-68,5) und im Jahre 2016 von 55 (IQR 45-66) auf, wohingegen die Gesunden im Median bei 64 (IQR 56-69) lagen (**Originalarbeit IV.**). Bei der Abfrage der Lebenseinstellung zeigte sich im Pessimismus Subscore kein Unterschied zwischen iTTP-Patienten und Kontrollen, dagegen waren die Patienten im Optimismus Subscore signifikant schlechter (2015 $p=0,011$; 2016 $p=0,006$). Im Zusammenhang mit der Depression konnten wir zeigen, dass die Resilienz der Patienten signifikant mit der Schwere der Depression korreliert ($p<0,0001$). Je widerstandsfähiger die Patienten abschnitten, desto geringer waren die Depressionen (**Originalarbeit IV.**). Die Beobachtungen decken sich mit denen anderer Autoren, die nahelegen, dass die Resilienz jedes Einzelnen mitentscheidend ist, ob er oder sie eine Depression entwickelt und das unabhängig von der Erkrankung (97, 98).

Entscheidend für die Entwicklung der Depression scheint auch die Anzahl der Erkrankungen. Dabei spielt es offensichtlich keine Rolle, um welche Erkrankung es sich handelt. Partnerschaft, Geschlecht, Alter und körperliche Fitness hatten hingegen keinen Einfluss auf die Entstehung der Depression. Andere Arbeitsgruppen (88, 99, 100) beschreiben bei iTTP-Patienten eine eingeschränkte Lebensqualität. Dies konnten wir in beiden Befragungen ebenfalls nachweisen und darüber hinaus belegen, dass die Lebensqualitätsminderung signifikant mit der Schwere der Depression zusammenhängt ($p<0,0001$) (**Originalarbeit IV.**).

Neben der eingeschränkten Lebensqualität birgt der hohe Prozentsatz an Depressionen und Angstzuständen bei iTTP-Patienten noch ein ganz anderes Risiko, nämlich das der erhöhten Morbidität und Mortalität. Untersuchungen zufolge kann bei Depressionen ein 2,4fach erhöhtes Mortalitätsrisiko (101) angenommen werden, das nicht abhängig von der Schwere der Depression zu sein scheint (102). Unter diesem Gesichtspunkt ist eine Behandlung der TTP Patienten, die bereits durch die immer wiederkehrenden akuten Schübe ein hohes Risiko haben, zwingend notwendig.

Nachdem die iTTP klinisch oft mit neurologischen Symptomen einhergeht, die sich von Kopfschmerzen über Sprachstörungen bis hin zu Bewusstseinstrübung und Krampfanfällen erstrecken, ist die Frage der neurologischen Langzeitfolgen naheliegend. Um die kognitiven Beeinträchtigungen der Patienten im Alltag nach einem akuten Schub zu beurteilen, haben wir uns für den subjektiven FLei-Fragebogen entschieden, der Gedächtnis, Aufmerksamkeit und Exekutivfunktionen umfasst (103) (**Originalarbeit II. und IV.**).

Der Gesamtscore hinsichtlich der geistigen Leistungsfähigkeit, sowie die drei Sub-Scores waren bei iTTP-Patienten im Vergleich zu den erhobenen zwei gesunden Kohorten, als auch der gesunden Literaturkohorte (103) signifikant schlechter (Abb. 9). Dieses Ergebnis deckt sich mit dem von Kennedy et al. (104) und Cataland et al. (99), die ebenfalls eine beeinträchtigte kognitive Leistungsfähigkeit bei iTTP-Patienten beschreiben. Beide Arbeitsgruppen haben die kognitive Beeinträchtigung mit Hilfe klinischer Bewertungsmethoden festgestellt, anstelle von Selbstberichten, wie wir sie verwendet haben. Beim Vergleich mit depressiven Patienten (aus der Literatur (103)) schnitten iTTP-Patienten signifikant ($p<0,001$) besser ab (**Originalarbeit II.**).

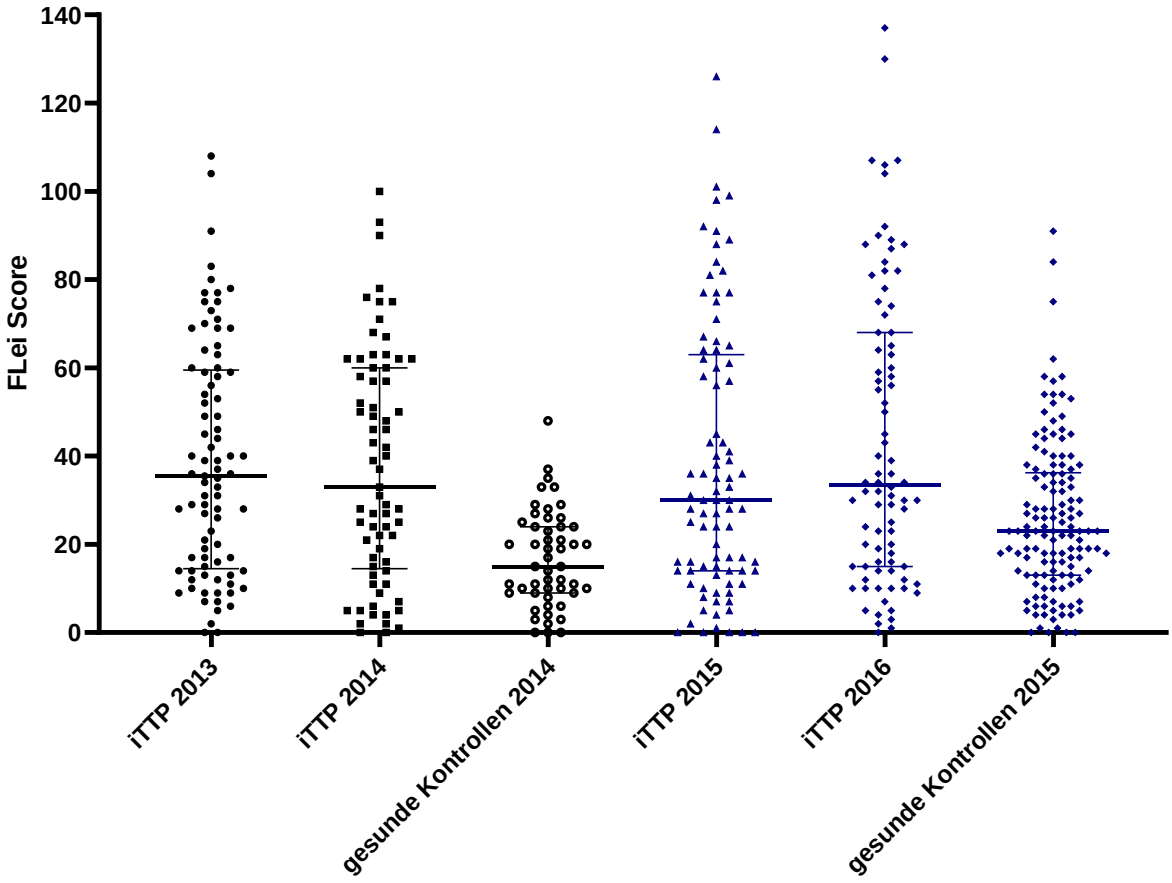


Abb. 9: Der FLei Score lag im Median 2013 (n= 85) bei 35,5 (IQR 15-60), 2014 (n= 69) bei 33 (IQR 15-60), 2015 (n= 85) bei 28,0 (14-60,5) und 2016 (n= 81) bei 34 (IQR 17-68). Die gesunde Kontrollgruppe 2014 lag im Median bei 15 (IQR 9-24) und die Kontrollgruppe 2016 wies einen Median von 22 (IQR 13,75-34,25) auf. Damit schnitten iTTP-Patienten signifikant schlechter in allen vier Befragungen ab (2013: p<0,001; 2014: p<0,01; 2015: p=0,008 und 2016: p<0,0001).

Bei den 60 (58%) iTTP-Patienten, bei denen der genaue Krankheitsverlauf erhoben werden konnte, wurde der Zusammenhang zwischen Erkrankung und geistiger Leistungsfähigkeit untersucht. Es war keine Korrelation zwischen der kognitiven Defizienz (FLei-Score) und der Anzahl der akuten iTTP-Schüben nachweisbar (r_S= 0,115) und auch der Vergleich von iTTP-Patienten mit einem versus zwei versus drei und mehr akuten Episoden war nicht signifikant verschieden (Kruskal-Wallis-Test, p= 0,078). Erstaunlich war aber vor allem, dass kein Zusammenhang zwischen neurologischen Symptomen während des akuten Schubes und der kognitiven Leistung ausgemacht werden konnte (**Originalarbeit II.**). Der mediane FLei Score bei Patienten ohne neurologische Symptome während der akuten iTTP betrug 31 (IQR 13-40), und

der mediane FLei Score bei Patienten mit neurologischen Symptomen während der akuten iTTP betrug 45 (IQR 15-65; Mann-Whitney-U-Test, p= 0,193, NS).

Bei der Gegenüberstellung der geistigen Leistungsfähigkeit und dem Ausmaß der Depression ergab sich eine hochsignifikante Korrelation. Sowohl in der ersten Erhebungsrunde mit 84 iTTP-Patienten (r_s= 0,643) als auch in der zweiten mit 69 iTTP-Patienten (r_s= 0,779) war ein signifikanter Zusammenhang (p< 0,001) zu sehen (**Originalarbeit II.**). Bei der Betrachtung aller 101 unterschiedlichen iTTP-Patienten (r_s= 0,696) war dies ebenfalls zu belegen (p< 0,001). Beim Vergleich der FLei Ergebnisse von iTTP-Patienten ohne klinisch relevante Depressionen (IDS-SR ≤25) mit gesunden Kontrollen schneiden beide Gruppen ähnlich gut ab. Auf der anderen Seite ist kein Unterschied hinsichtlich des FLei Scores von iTTP-Patienten mit klinisch relevanten Depressionen (IDS-SR >25) gegenüber depressiven Kontrollen auszumachen. Dieses Ergebnis wirft die Frage auf, ob die Beeinträchtigung der kognitiven Leistungsfähigkeit nicht vielleicht doch eine direkte Folge der Depression und nicht das Ergebnis einer vorangegangenen zerebralen mikrovaskulären Thrombose und Ischämie ist, wie Kennedy et al. (104) postulierten

Dass es einen Zusammenhang zwischen Depressionen und relevanten kognitiven Problemen gibt, ist in der Literatur beschrieben (105). Und die Tatsache, dass iTTP-Patienten ohne klinisch relevante Depressionen im FLei Score vergleichbar mit Gesunden abschneiden, und dies unabhängig von der Schwere der iTTP-Erkrankung, stützt diese These.

Andererseits fanden Han et al. (85) keinen Zusammenhang zwischen Depression und kognitiver Leistung bei iTTP-Überlebenden. Allerdings wurde hier der Beck Depression Inventory, ein Fragebogen zur Selbstabschätzung depressiver Symptome, mit einer psychiatrischen Bewertung der kognitiven Leistung verglichen. Somit untermauern die Ergebnisse von Han und Kollegen (85) unsere Hypothese, dass die selbst eingeschätzte kognitive Beeinträchtigung in erster Linie auf die Depression zurückzuführen ist. Inwieweit die Verringerung der kognitiven Leistungsfähigkeit durch mikrovaskuläre Thrombosen während akuter iTTP-Schübe verursacht wird und inwieweit die beeinträchtigte kognitive Leistungsfähigkeit eine direkte Folge der Depression ist, ist unklar und muss weiter prospektiv untersucht werden**.**

5. Zusammenfassung

Im Rahmen dieser Habilitationsschrift wurde der Krankheitsverlauf der iTTP am damals größten in Deutschland existierenden Patientenkollektiv in retrospektiven und prospektiven Studien untersucht.

Kernergebnisse unserer Studien sind:

Die iTTP ist keine rein akute Erkrankung, sondern eine chronische Erkrankung mit potentiell schweren Langzeitfolgen.

Patienten sind nachhaltig in ihrem Leben belastet und eingeschränkt. Folglich bedürfen sie neben der Akutbehandlung einer längerfristigen Nachsorge. Vor allem leiden die Patienten unter zum Teil schweren Depressionen und Angstzuständen. Darüber hinaus weisen iTTP-Patienten eine verminderte kognitive Leistungsfähigkeit gegenüber Gesunden auf, deren Ausmaß signifikant mit der Schwere der Depression korreliert. Interessanterweise ist kein Zusammenhang zwischen der Entwicklung von Depressionen und der Schwere sowie der Anzahl der akuten Schübe nachweisbar. Des Weiteren ist die Resilienz der iTTP-Patienten geringer ausgeprägt als die von Gesunden und die Lebensqualität, sowie die Lebenseinstellung, nachhaltig schlechter.

Retrospektiv wie prospektiv wurde die Pathophysiologie der iTTP verfolgt.

Die Studiendaten beweisen, dass ein akuter iTTP Schub zwingend mit einem massiven Abfall bzw. einer deutlich verminderten ADAMTS13-Aktivität (<10%) einhergeht. Allerdings ist auch ersichtlich, dass Patienten mit konstant niedrigem ADAMTS13 nicht zwingend einen Schub erleiden.

Das Substrat der ADAMTS13, der VWF, welcher in diesem Zusammenhang relativ wenig untersucht ist, muss mehr im Blickpunkt stehen. Im Rahmen der Originalarbeit VI konnte eine neue VWF Multimer Ratio etabliert werden. Das Verhältnis zwischen hochmolekularen zu niedermolekularen VWF Multimeren spiegelt das in vivo bestehende Zusammenspiel zwischen ADAMTS13 und dem VWF wider.

Vor akuten Schüben verschiebt sich der Anteil der VWF Multimeren zu den hochmolekularen, folglich steigt der Quotient an. Zu Beginn des Schubes fällt er deutlich ab, was auf den Verbrauch in den Mikrothromben zurückzuführen ist. Überraschend ist, dass einige Tage bis Wochen vor einem akuten iTTP-Rezidiv eine signifikant höhere VWF MM Ratio nachzuweisen war, als bei den Patienten, die sich in Remission über den ganzen Studienzeitraum befanden, und das unabhängig davon, ob die ADAMTS13-Aktivität <10% oder >10% lag. Darüber hinaus war die ADAMTS13-Aktivität Tage bis Wochen vor dem akuten Schub nicht zwingend <10%. Die Hypothese, dass Entzündungen einen akuten Schub triggern, da sie die Spaltung des VWF durch ADAMTS13 vermindern, kann in diesem Fall nicht bestätigt werden. Patienten die im Beobachtungszeitraum keine akuten iTTP-Schübe hatten, haben hingegen konstante VWF MM Ratio Werte.

An unserem retrospektiven Patientenkollektiv kann nicht belegt werden, dass sich die Gabe von Rituximab signifikant positiv auf die Schubschwere oder auf die Rückfallrate auswirkt. Allerdings ist ein Trend zu kürzerer Schubdauer und zu weniger Frührezidiven, binnen des ersten Jahres, nach einem akuten Schub, erkennbar.

Die klinische Einteilung der Schübe nach ihrer Schwere ist an retrospektiven Patientenkollektiven etabliert worden und bestätigt die These, dass Initialschübe die schwerwiegendsten Verläufe aufweisen.

Insgesamt sind Männer deutlich seltener von der iTTP betroffen, dafür legen die Daten nahe, dass sie deutlich schwerer an der Erkrankung leiden mit einer signifikant höheren Rückfallrate als Frauen.

Mikrothrombosen, insbesondere im Lungenkreislauf, bei Patienten mit COVID-19, führten zur Hypothese, dass es sich hierbei um eine thrombotische Mikroangiopathie handeln könnte. Wir haben in einem Mainzer Kollektiv keinen Anhaltspunkt dafür gefunden, dass COVID-19 eine klassische thrombotische Mikroangiopathie ist.

6. Literatur

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7. Wissenschaftliche Originalpublikationen

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Falter T, Alber KJ, Scharrer I. Long term outcome and sequelae in patients after acute thrombotic thrombocytopenic purpura episodes. Hamostaseologie. 2013 May 29;33(2):113-20.

Long term outcome and sequelae in patients after acute thrombotic thrombocytopenic purpura episodes*

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Keywords
Thrombotic thrombocytopenic purpura, ADAMTS13 activity, long term outcome, neurocognitive impairment

Summary
We report on 21 patients with idiopathic thrombotic thrombocytopenic purpura (TTP) whose courses of disease have been followed from the respective diagnosis until now. They had a documented ADAMTS13 activity below 5%, a high autoantibody titer and detectable ultralarge von Willebrand factor (VWF) multimers during their episodes. The initial diagnosis was based on clinical symptoms and on laboratory parameters: thrombocytopenia, haemolytic anaemia, schistocytes and an increased LDH level. 103 acute clinical episodes of 21 TTP-patients during a time period of 30 years are described. Case histories, comorbidities and sequelae were retrospectively documented. Results, conclusion: Although patients are consistently in a prothrombotic status, clinical acute manifestations only occur after triggering. Most common trigger factors are gastrointestinal infections and pregnancy. The relapse risk per month is 0.026; men have a higher risk for relapses (0.044) than women (0.021). Patients recover physically well, except for renal insufficiency in four cases. Nevertheless, major portion of patients suffers persistently from depression, anxiety disorders and persistent neurocognitive impairments.

Schlüsselwörter
Thrombotisch thrombozytopenische Purpura, ADAMTS13 Aktivität, Spätfolgen, neurokognitive Störungen

Zusammenfassung
Wir berichten über 21 Patienten mit idiopatischer thrombotisch thrombozytopenischer Purpura (TTP), deren Krankheitsverlauf von der Diagnose bis heute verfolgt wurde. Die Patienten wiesen zum Zeitpunkt der Schübe eine ADAMTS13-Aktivität unter 5%, hohe Autoantikörpertiter und ultralange VWF-Multimere auf. Die initiale Diagnose erfolgte aufgrund klinischer Symptome und Labordaten: Thrombozytopenie, hämolytische Anämie, Schistozyten und LDH. Insgesamt werden 103 Schübe von 21 TTP-Patienten über einen Zeitraum von 30 Jahren beschrieben. Krankheitsverlauf sowie Begleiterkrankungen und Folgen der TTP wurden retrospektiv erfasst. Ergebnisse, Schlussfolgerungen: Obwohl die Patienten sich in einem dauerhaft prothrombotischen Status befinden, treten TTP-Schübe meist infolge zusätzlicher Auslöser, insbesondere gastrointestinaler Infektionen sowie Schwangerschaften, auf. Das Risiko eines Rückfalls des Kollektivs beträgt 0,026 pro Monat, wobei Männer ein höheres Rückfallrisiko aufweisen (0,044) als Frauen (0,021). Bis auf vier Patienten mit chronischer Niereninsuffizienz, erholten sich die Patienten körperlich gut. Dennoch leidet ein Großteil der Patienten unter Depressionen, Angststörungen und neurokognitiven Defiziten.

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Krankheitsverlauf und Spätfolgen bei Patienten nach akuten Schüben der thrombotisch thrombozytopenischen Purpura
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Thrombotic thrombocytopenic purpura (TTP) is a relatively rare thrombotic microangiopathy (TMA) characterized by

- microvascular thrombi,
- non-immune haemolytic anaemia and
- consumptive thrombocytopenia (1, 2).

These symptoms are due to a decrease or complete deficiency of ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 motifs 13), which cleaves high molecular and prothrombotic von Willebrand factor (VWF) multimers in less active molecules (3, 4). The ultralarge and prothrombotic VWF multimers initiate spontaneous VWF-platelet aggregation, thus leading to microvascular thrombi in arterioles and capillaries of different organs (5). TTP patients present with many different symptoms. Typical clinical symptoms:

- neurologic abnormalities like headache, vigilance dysfunction, coma, seizures,
- petechiae,
- abdominal pain,
- nausea, vomiting,
- diarrhea,
- fever,

laboratory characteristics (patients n = 21)			acute phase	remission
abnormalities	thrombocytopenia (<150/nl)		21	0
	schistocytes (>5‰)		21	0
	increased LDH (>250 U/l)		21	0
	decreased haemoglobin (<12 g/dl)		21	0
	ultralarge VWF multimers		21	fluctuating
specific parameters	ADAMTS13 activity	<5%	21	7
		>5 to 50%	0	5
		10 to 100%	0	5
	ADAMTS13 autoantibody	constant >2 BU	21	0
		fluctuating 0–2.5 BU	0	21

LDH: lactate dehydrogenase; VWF: von Willebrand factor; BU: Bethesda units

Tab. 1 Laboratory characteristics for acute phase of idiopathic TTP and remission

patients characteristics		
age (years) at first acute TTP episode		25.5 (12–53)
total number of acute episodes		103
number of relapses	0	2 (9.5%)
	1	5 (23.8%)
	2	2 (9.5%)
	3	4 (19.0%)
	4 and more	8 (38.0%)
	highest	12
	average	4.8
sex	female	16
	male	5
blood group	0	10
	A	9
	AB	0
	B	1
	not known	1
potential trigger factors	infections total	30
	• respiratory tract	10
	• gastrointestinal	10
	• urinary tract	5
	vaccination	2
	drugs*	5
	hormonal contraceptive	5
	pregnancy / postpartum	9
	parodontitis	2
additional autoimmune disease		10
• rheumatic disorder**		5
• Hashimoto thyroiditis		4
• idiopathic thrombocytopenia		4
• antiphospholipid syndrome		1
• multiple sclerosis		1
• Raynaud syndrome		1
allergies#		7

*malaria prophylaxis, quinine, acetylsalicylic acid ticlid, chemotherapy;
**psoriasis, SLE, chondritis, polymyalgia rheumatica;
#house-dust allergy (2), food allergy (3), sun allergy (1), nickel allergy (1), allergies against drugs (2)

Tab. 2 Baseline characteristics of all patients with idiopathic TTP (n = 21)

- sometimes haematuria, chest pain and weakness.

A rapid course of deterioration and death may occur without treatment of TTP (6).

Hereditary TTP caused by mutations in the ADAMTS13 gene is one of the primary TTP forms. The more common form is idiopathic TTP due to autoantibodies against ADAMTS13. Severe diseases (cancer, HIV, vasculitis) as well as drugs (chemotherapeutics) can cause secondary TTP (6).

Presently, plasma exchange is still the mainstay of therapy for clinically acute manifestations of TTP and reduced the mortality from 90% to 8–30% (7–10). In addition, patients with idiopathic TTP are treated with immunosuppressive drugs (cortisone, vincristine or rituximab) (6, 11).

> Aim of therapy is to achieve a durable remission with complete recovery.

Criteria for recovery are
- normal physical conditions and
- regular laboratory data especially platelets, haemoglobin and lactate dehydrogenase (LDH).

However, a higher risk of persisting neurocognitive impairments and depressions that may exist for TTP patients is discussed (12).

We report on long term outcome and sequelae of patients with severe ADAMTS13 deficiency, high autoantibody titers against ADAMTS13 and detectable ultralarge VWF multimers.

Patients, material, methods

We retrospectively describe the long term outcome and sequelae of 21 patients from different nationwide clinics with 103 acute clinical episodes of TTP over 30 years. Patients were recruited of a collective of TTP patients who have been completely observed from first manifestation until now. Therefore, medical reports and records of each patient were analyzed. The medical records/reports come either from the TTP-treating haematologists or from a psychia-

trist. Before recruiting all TTP patients underwent an examination at the University Medical Center Mainz and filled in a questionnaire.

Their blood samples were investigated for ADAMTS13 activity, ADAMTS13 antibodies and ultralarge VWF multimers. A full blood cell count was conducted as well. To concretize the patient collective and minimize heterogeneity only idiopathic TTP patients with a documented ADAMTS13 activity below 5%, a high autoantibody titer >2 Bethesda units (BU) and detectable ultralarge VWF multimers at the time of acute episode were chosen. The severe ADAMTS13 activity (<5%) appears to be specific for TTP in contrast to a low ADAMTS13 activity (<10%). Patients with liver cirrhosis and sepsis for example can also show an ADAMTS13 activity below 10% (13). The autoantibodies against ADAMTS13 are specific for idiopathic TTP.

The initial diagnosis of TTP was made based on clinical symptoms and laboratory parameters. Diagnostic criteria were thrombocytopenia, non-immune haemolytic anaemia, schistocytes and an increased LDH level, along with diverse clinical features (▶Tab. 1). Since 2004, it has been possible to measure ADAMTS13 activity and antibodies reliably in our laboratory for the definite diagnosis of TTP. With these methods the diagnosis of all patients could be confirmed.

Check-up of laboratory data was performed by the respective clinics. ADAMTS13 activity and ADAMTS13 antibodies have been monitored in the Institute of Clinical Chemistry and Laboratory Medicine University at the Medical Center of the Johannes Gutenberg University Mainz in acute phase as well as in remission. First, the parameters were investigated with BCS-method according to Böhm (14). In 2010, the method was changed to a commercial FRETS-VWF73 assay (American Diagnostica GmbH, ACTIFLUOR™ ADAMTS13 activity kit).

In remission, all patients showed fluctuating ADAMTS13 autoantibody titers from 0 to 2.5 BU. Ultralarge VWF multimers were always detectable. The ADAMTS13 activity of five patients reached nearly normal values in remission. Five patients showed fluctuating ADAMTS13 activities between 5 to 50%, and seven patients had constant ADAMTS13 activities below 5% without developing an acute episode (▶Tab. 1).

Disease history was investigated of all patients from the first manifestation until now. We documented number, frequency, duration, symptoms and severity, trigger factors, laboratory data and treatment of each acute clinical episode. Furthermore, we collected data about other autoimmune diseases, allergies and blood groups. In a separate questionnaire we ask patients for sequelae and life quality since diagnosis or acute clinical episodes.

Remission of an acute clinical episode was defined by platelet count (≥150/nl) and LDH (≤250U/l) normalization for at least 3 days and a start of hemoglobin increase. Therapy was prescribed by the attending physician. To achieve remission main treatment was plasma exchange (PEX), typically 1 to 1.5 volumes of PEX daily. Immunosuppressive drugs were often applied frequently as well. An iterative drop of platelets after three days of remission was diagnosed as an early recurrence and counted as full relapse. The relapse risk is defined as an event rate calculated as follows: (total number of events) / (total follow-up time).

Tab. 3 Clinical presentations of acute episodes of TTP: All clinical features are summarized of 81* acute episodes of 21 patients. (* Of these acute episodes detailed clinical data were available.)

clinical characteristics		patients (n = 21)
severe neurological abnormalities	coma / collapse	10
	seizures	8
	stroke	6
	aphasia	15
	paresis / ataxia	11
minor neurological abnormalities	headache	9
	vision disorders	8
	vertigo	7
	fatigue / exhaustion	12
	confusion / disorientated	7
	paresthesias	8
dyspnea / atelectasis		8
myocardial infarction		2
renal insufficiency / dysfunctions of urinary passages		9
haematuria		9
icterus		5
petechiae		13
haematoma		13
bleedings		9
thrombosis		2
pallor of skin and mucosa		4
weakness		7
fever / chills and fever		9
gastrointestinal symptoms (nausea, vomiting, diarrhea)		13
abdominal pain		7
others	splenomegaly, multiple organ failure, surdity, joint pain	
full pentad (11): renal insufficiency, neurological symptoms, fever, anaemia, thrombocytopenia		2#

in patient 5 and 8 once in each case

Results

Patients

All 21 patients we monitored showed an ADAMTS13 activity of less than 5% and more than 2 BU of ADAMTS13 autoantibodies as well as ultralarge VWF multimers in plasma in the acute phase of TTP (▶Tab. 1). We included 16 (76%) women and 5 (24%) men aged from 17 to 70 years. Among these 21 patients 10 belong to

Tab. 4 Course of disease and treatment

case / patient	acute episodes					treatment		
	first	course	last	total number	time period	PEX	additional drugs	additional therapy
1	1982	1992 – 1993 – 1998 – 1998 – 1998 – 2003	2012	8	30 years	yes	cortisone	IVIG
2	2003	2003	2004	3	9 years	yes	cortisone, vincristine	
3	2004	2004 – 2004 – 2005 – 2007 – 2010	2012	7	8 years	yes	cortisone, rituximab	
4	2008			1	4 years	yes	cortisone	
5	2006	2006 – 2008 – 2011 – 2011 – 2011	2012	7	6 years	yes	cortisone, rituximab, levetiracetam, valproic acid	
6	1992	2001 – 2002 – 2005 – 2006 – 2007 – 2010	2012	8	20 years	yes	cortisone, rituximab, gabapentin, phenytoin	IVIG
7	2005		2008	2	7 years	yes	cortisone, rituximab	
8	2000	2001 – 2009	2012	4	12 years	yes	cortisone, vincristine, ASA, rituximab, phenytoin	
9	1994	2000 – 2002 – 2005 – 2006 – 2008 – 2009 – 2010	2011	9	18 years	yes	cortisone, ASA	
10	1997	1998 – 1999 – 1999 – 2000 – 2001 – 2006 – 2007 – 2010 – 2011 – 2012	2012	12	15 years	yes	cortisone, ASA, rituximab	splenectomy, insulin
11	2008		2012	2	4 years	no	cortisone, vincristine, rituximab, erythropoietin, phenytoin	IVIG
12	1997	2001 – 2004	2009	4	15 years	yes	cortisone, vincristine, rituximab, cyclophosphamide, azathioprine, phenytoin	immune adsorption, red blood cell-and platelet-concentrate
13	1993		2004	2	19 years	yes	cortisone	
14	1990	1991 – 1991	1992	4	22 years	yes	cortisone, vincristine, ASA, phenytoin	
15	2002	2003 – 2003 – 2004 – 2009 – 2009 – 2009 – 2010	2010	9	10 years	yes	cortisone, rituximab	
16	2010		2011	2	2 years	no	cortisone	FFP, IVIG, red blood cell-concentrate
17	1983	1986 – 1990 – 1993 – 2003 – 2006 – 2008	2012	9	29 years	yes	cortisone, rituximab	splenectomy, platelet-concentrate
18	1996	2004 – 2006	2012	4	16 years	yes	cortisone, vincristine	
19	2012	2012	2012	3	1 years	yes	rituximab	
20	2008		2010	2	4 years	yes	cortisone, rituximab	insulin
21	2010			1	2 years	yes	cortisone	

PEX: plasma exchange, IVIG: intravenous immunoglobulin therapy; ASA: acetylsalicylic acid; FFP: fresh frozen plasma

blood group 0 and nine to blood group A. Only one patient belongs to blood group B and no one to blood group AB. Patient 14 did not state her blood group and refused PEX and other blood concentrates because of her religious denomination.

TTP was first diagnosed at the average age of 25.5 years. The youngest patient was 12 years and the oldest 53 years at time of diagnosis. All 21 patients have had a total number of 103 acute clinical episodes over a time period of 30 years. Average number of relapses per patient is 4.8 and highest number of relapses is 12. Two patients had only one acute manifestation without relapse until now. 38% had four and more acute clinical manifestations (▶Tab. 2).

Clinical features of TTP present in many different symptoms; only two times with the full TTP-pentad (10) (▶Tab. 3). Neurological symptoms are predominant and vary in intensity from severe neurological symptoms (seizures, coma and apha-

sia) to minor neurological symptoms (headache, fatigue, paresthesia). Moreover, very typical clinical features are different types of bleeding, petechiae, haematoma and epistaxis. Blood in stool and haematuria are stated often, too. Resulting from haemolytic anaemia icterus, pallor and weakness were present. Gastrointestinal symptoms like nausea, vomiting and diarrhea as well as abdominal pain and dyspnea are unspecific but abundant clinical presentations for TTP (▶Tab. 2, ▶Tab. 3).

It is known that different factors induce an acute onset of TTP. Most frequent trigger factors are infections, especially gastro-intestinal infections. Use of contraceptives as well as pregnancy and the postpartum period are also often stated (▶Tab. 2).

In the presented collective seven pregnancies are documented. Four patients have had one pregnancy with at least one acute episode. Patient 1 and 15 had even three acute episodes in their pregnancies. Patient 13 had one relapse during her first pregnancy and she was prophylactically treated with FFP in her subsequent two pregnancies. Patient 4 and 20 were diagnosed for TTP during pregnancy and for patient 4 this has been the only acute phase of TTP without relapse for 10 years until now. Patient 20 had only one mild relapse in four years. In two cases labor had to be induced because of acute TTP episodes.

In addition to TTP, 10 patients suffer from other autoimmune diseases. One patient suffers from four autoimmune diseases (SLE, psoriasis, MS, Raynaud syndrome). Four patients have two additional autoimmune diseases. Six patients have one further autoimmune disease (▶Tab. 2). Common allergies against house-dust, food and pollen are also stated seven times (▶Tab. 2).

Patient history

A collective of 21 patients was retrospectively observed from time of first manifestation until now. Most of them have been treated for TTP for 10 to 20 years and three patients have suffered from TTP for more than 20 years; six patients were diagnosed with TTP in the last five years and four patients were diagnosed in the last nine years (▶Tab. 4).

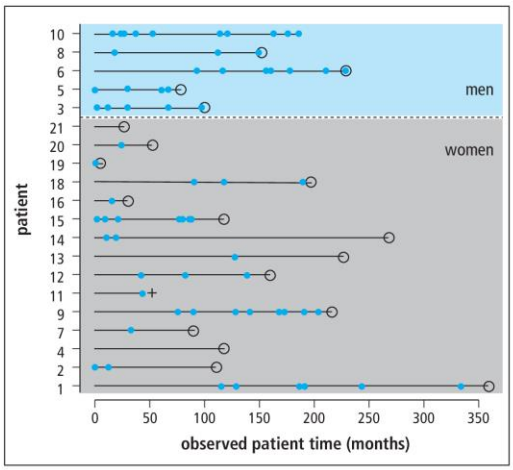

Fig. 1
Event history:
All relapses of 20 idiopathic TTP patients since manifestation until November 2012 (*For patient 17 no exact data was ascertained.)

Altogether these 21 patients had 103 bouts in 253 years. We have found 14 patients who have suffered seven and more acute episodes in six to 30 years and 13 patients with less than five acute episodes in one to 22 years. Seven patients (number 1, 2, 3, 5, 10, 14, 15) had two and more acute episodes in rapid succession. The relapse risk per month for these 21 TTP patients is 0.026. The five men in our collective have a higher risk for relapses (0.044) than the 16 female patients (0.021) (▶Fig. 1).

However, no recognizable regularity/tendency in terms of distribution for the whole collective can be observed; therefore every patient has to be monitored independently of each other (▶Tab. 4).

Treatment

All except two patients were treated with PEX during at least one acute clinical manifestation. Patient 11 refused PEX and other blood concentrates because her religious denomination does not approve this kind of therapy (witness of Jehova) but she was treated with immunoglobulines (IVIG) and erythropoietin. She died from her first relapse due to refusing treatment. Patient 16 responded well to FFP, red blood cell-concentrates in combination with cortisone and IVIG in both clinical episodes. Patients 1, 6, 7, 8, 10 and 12 were not treated with PEX in every acute phase, especially in their first manifestation or in mild acute episodes.

Only two patients have never been treated with cortisone. Rituximab was used for 11 patients, ten times in combination with cortisone. Five patients were further treated with vincristine always combined with cortisone and two times with rituximab. One patient was treated in her first acute episode with cortisone, vincristine, cyclophosphamide and azathioprine in combination with immune adsorption as well as red blood cell-concentrates. PEX was not conducted and TTP was not diagnosed at this time point.

Six patients additionally required anticonvulsives. Phenytoin was used five times, one time in combination with gabapentin. One patient was treated with a combination of levetiracetam and valproic acid. Other additional drugs are acetylsalicylic acid (ASA) (four times), intravenous immunoglobulin therapy (IVIG) (three times) and Insulin (two times), the latter only due to side effects of cortisone. Splenectomy was conducted in patient 10 at fifth relapse and patient 17 at clinical manifestation without long-term success.

Sequelae

Data for sequelae of TTP patients were collected with the help of medical reports and patients` own statements. The medical re-

ports either come from a psychiatrist or from the TTP- treating haematologists.

Ten patients have been treated due to depression and many of them needed psychological help. One patient has been under in-patient psychiatric treatment. Furthermore, many patients complain of anxiety disorders. As a result of depression and anxiety disorders these patients suffer from general fatigue, exhaustion, lack of drive, adjustment disorders, social anxiety disorder and chronic insomnia. Neurocognitive impairments including brain-fag syndrome, lack of concentration, amnesic aphasia and dysphasia, disturbance of long-term and short-term memory occur frequently as well.

Six patients are afflicted with epilepsy as a sequelea of stroke which they suffered from during their bouts. Four patients developed renal insufficiency. One patient died during first relapse. Many of the patients suffer from side effects of cortisone and other immunosuppressive drugs, especially from Cushing's syndrome, cortisone acne, overweight, osteopenia and alopecia. Most of the patients stated that they lead a retired life with a reduced quality of life, still emphasizing problems in their daily life, social communication and relationships; their professional life is impaired right up to a temporary or permanent disability.

Discussion

It is well known that ADAMTS13 activity below 5%, presence of autoantibodies and ultralarge VWF multimers are not the only reasons for an acute TTP episode. Additional trigger factors are needed to cause clinical TTP episodes (15, 16).

Pregnancy attracts attention as very strong trigger for TTP (17). All of our patients followed up here who have children had at least one acute episode during their pregnancy. We could observe eight acute episodes in seven pregnancies out of five patients. Furlan and Lämmle also describe pregnancy as an important trigger and report of two congenital and four acquired TTP cases in this context (18). Interestingly, the two women with congenital TTP both have brothers with congenital severe ADAMTS13 deficiency but without any acute phases of TTP. Here, we report on two patients who experienced their first TTP episode during pregnancy. Despite the fact that ADAMTS13 may be reduced in healthy pregnant women during third trimester we could not find a tendency for this trimester (19, 20). The TTP episodes extend over the complete childbearing period.

The most frequent triggers in this collective were infections especially GI-infections as also described by Furlan and Lämmle (18). The level of VWF, an acute phase protein, increases in cases of inflammation. VWF increase might be one causing factor in these patients with a prothrombotic state (21, 22). However, the mechanisms of different known trigger factors are not fully understood.

Our collective of 21 patients with severe ADAMTS13 deficiency, high antibody titers and ultralarge VWF multimers shows very heterogeneous clinical symptoms and courses of disease. The diversity of clinical symptoms is comparable to the Oklahoma TTP – haemolytic uremic syndrome (HUS) Registry (23) and the Regional UK TTP Registry (24). The symptoms range from mild bleeding disorders, abdominal pain, GI-symptoms to severe neurological disorders. We report on only eight acute episodes with renal failure but 87 different neurological symptoms, probably because of our specific idiopathic TTP collective (25). Neurological symptoms and renal failure occur nearly in the same frequency in the Oklahoma TTP-HUS Registry.

The complete pentad (10) of clinical features was only presented in two of 103 acute episodes. In another two episodes, only renal dysfunction was missing. The pentad was defined in 1966 by Amorosi and Ultmann, and at this time point knowledge of the pathophysiology of TTP was only moderate. Thus it was hardly possible to clearly distinguish between TTP and HUS, which might be the reason why renal dysfunction is not often observed for TTP anymore. Neurological symptoms often occurred. In some cases, severe clinical symptoms probably could be prevented by a close-meshed control of blood count and early recognition of platelet decrease which lead to an immediate onset of therapy (PEX). It seems that the age of first manifestation has no influence on degree of severity and recurrence rate. Patient 10 and 18 were 50 and 53 years at time of TTP diagnosis but patient 10 has already 12 relapses whereas patient 18 has only four relapses until now. The youngest patient of our collective was 12 years old at first manifestation and has only two relapses in seven years in contrast to patient 15 with nine relapses in 10 years since the age of 17.

It is striking that four of only five men in our collective had seven and more relapses including patient 10 with the highest recurrence rate of the monitored group. Kremer-Hovinga also reported that mostly men are significantly associated with relapse (26). Our male patients experienced even more severe acute episodes in contrast to the women.

Especially the first manifestation seems to be the most severe acute episode. Probably this can be attributed to the fact that the diagnosis was not obvious and therefore the appropriate treatment could not be started directly. Plasma exchange (PEX) is still treatment of choice until now. However, two of 21 patients in our collective did not receive PEX at any time. But it has to be mentioned that we have to differentiate between patient 11 and patient 16 as patient 11 refused all blood products because of religious ideology. Patient 16 had only two mild acute episodes and responded well to FFP. Patient 1, 6, 7, 8, 10 and 12 were not treated with PEX in every acute phase.

Splenectomy is discussed as a possible therapy especially for patients with many relapses and poor response to treatment with PEX (27, 28). Spleen was removed in two patients of our collective; in patient 17 splenectomy, which was performed at manifestation, seems not to have a sustained positive effect for long-term course of disease. This patient has already had eight relapses in 29 years but after all the average remission duration is between three and four years with one remission of even 10 years. It is difficult to determine whether this long-term course is the result of splenectomy. Patient 1 has had almost the same number of relapses in the same time period but without splenectomy. Patient 10 had the longest time of remission (five years) directly after splenectomy in his

fifth relapse. Interestingly, this course of TTP was also described by Crowther et al. who observed the outcome of splenectomy in six patients (29). Only one patient was monitored for more than five years and just this patient relapsed in his fifth year of remission. Duration of observation can prove a problematic issue; splenectomy might prolong the period of remission but most of the studies describe cases with a follow-up of only five years. There is only one case reported with no relapse within eight years after splenectomy (27).

> Immune suppression is an adequate and a common therapy for idiopathic TTP alone as well as in combination with PEX (30).

Since market introduction in 1998 rituximab has been in off-label use for immune suppressive treatment in TTP. Also in our collective 12 patients were treated with rituximab. But the application was inhomogeneous so that a clear statement on an efficacy is not possible. Steroids which were administered in almost all 103 acute episodes are controversially discussed in the literature (31). In patient 11 rituximab without PEX was not sufficient for survival. In five cases of severe as well as mild acute episodes rituximab was successfully applied as monotherapy. Other authors state that rituximab could be used as alternative treatment of TTP as well as prophylactic therapy (32–35). Patients achieved a clinical remission under therapy but ADAMTS13 activity did not increase to more than 10% and ADAMTS13 autoantibody were still detectable in contrast to other studies (33, 34, 36). Altogether, administration of rituximab was so heterogeneous that a clear statement about efficacy is not possible.

Even though the long-term outcome is unspecific and varies widely in number of relapses and severity of bouts, fortunately 20 patients with 101 acute TTP episodes were successfully treated. Certainly, this is due to a close-meshed control of blood counts leading to early recognition of acute episodes and timely start of treatment. Zheng et al. also concluded a low mortality for idiopathic TTP but a high relapse rate if ADAMTS13 autoantibodies are detectable (37). In our collective there are two patients (9.5%) without relapse; the main part had at least one relapse. However, the number of patients with only one or two acute episodes and the number of patients with seven acute episodes and more is nearly the same. Therefore we cannot conclude from our small study, that those patients with lower ADAMTS13 activity than 5% and autoantibodies have a higher risk for relapses.

Chronic relapsing and life-threatening diseases are often associated with psychological disorders (38). It is also described that TTP patients suffer from depression in combination with adjustment disorders as well as social anxiety disorder. Most of the patients have neurocognitive impairments including brain-fag syndrome, exhaustion, general fatigue combined with lack of concentration, amnesic aphasia and disturbance of long-term and short-term memory (13, 39, 40). This is comparable to the sequelae of our investigated patients. Many of them complained about fears of the future. On the one hand they are afraid of social relationships, family planning and changing lifestyle. On the other hand they are limited in their professional life in terms of physical and cognitive efficiency.

A further problem is the difficult and long lasting therapy. In addition side effects of immunosuppressive therapy frequently occur sometimes resulting in bad compliance.

As can be seen, our nationwide collective reflects the heterogeneous nature of idiopathic TTP cases. Neurological symptoms are predominant and the full TTP-pentad only occurred two times in 103 acute episodes. This reflects that the complete pentad of symptoms is not critical for diagnosis of TTP.

> The relapse risk per month is 0.026 whereas men have a higher relapse risk (0.044) than women (0.021).

Due to the limited number of patients this statement is not representative and should be proven. Moreover, men seem to have the more severe clinical acute episodes. In many cases a close connection with trigger factors, especially infections and pregnancy, was obvious.

Conclusion

TTP-patients recover well from their acute phases with regard to physical examinations and laboratory data despite ADAMTS13 activity and inhibitor. However, many of them suffer from neurocognitive impairments, anxiety disorders and depressions. As a result they have to cope with a durable restriction of their daily routine both in private as well as in professional life.

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Conflict of interest

The authors declare that they have no conflict of interest.

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7.2. Originalarbeit II.

Falter T, Schmitt V, Herold S, Weyer V, von Auer C, Wagner S, Hefner G, Beutel M, Lackner KJ, Lämmle B, Scharrer I. Depression and cognitive deficits as long-term consequences of thrombotic thrombocytopenic purpura. Transfusion. 2017 May;57(5):1152-1162.

Depression and cognitive deficits as long-term consequences of thrombotic thrombocytopenic purpura

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BACKGROUND: Thrombotic thrombocytopenic purpura (TTP) is an acute life-threatening microangiopathy with a tendency of relapse characterized by consumptive thrombocytopenia, microangiopathic hemolytic anemia, and spontaneous von Willebrand factor–induced platelet clumping leading to microthrombi. The brain is frequently affected by microthrombi leading to neurologic abnormalities of varying severity.

STUDY DESIGN AND METHODS: The aim of this observational cohort study was to investigate the prevalence of depression and cognitive deficits in 104 patients having survived acute TTP. TTP survivors were repeatedly assessed by means of different standardized questionnaires to evaluate depression (IDS-SR) and mental performance (FLei). We received answers of 104 individual TTP patients and 55 of them participated in both surveys.

RESULTS: Seventy-one of the 104 responding TTP patients (68%) suffered from depression and the severity of depression was similar in both surveys performed 1 year apart. Furthermore, TTP patients had considerably lower cognitive performance than controls. There was no correlation between prevalence of depression and cognitive deficits and the number and the severity of acute episodes. Impairment of mental performance correlated with the severity of depression ($r_s = 0.779$).

CONCLUSION: The prevalence of depression and cognitive deficits was significantly higher in TTP patients. Cognitive impairment seemed to be a consequence of depression, almost independently of number and severity of TTP episodes.

Thrombotic thrombocytopenic purpura (TTP) is an acute, life-threatening disorder that is characterized by widespread von Willebrand factor (VWF)- and platelet (PLT)-rich microthrombi involving capillaries and arterioles of the brain and other organs.[1] Further characteristics are thrombocytopenia due to consumption of PLTs and microangiopathic hemolytic anemia with destruction of red blood cells.[2] The most common form of TTP is caused by inhibitory autoantibodies to the VWF-cleaving protease ADAMTS13.[1-3] Patients surviving an acute TTP bout and showing normalization of the laboratory variables are often considered

ABBREVIATIONS: FLei = German questionnaire for complaints of cognitive disturbances; IDS-SR = Inventory of Depressive Symptomatology, Self-Report; IQR = interquartile range; TTP = thrombotic thrombocytopenic purpura.

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to be cured; however, recurrences of acute TTP attacks are common.[4] In recent years the long-term consequences of this disease were noted because in spite of normal laboratory data, many TTP patients complained of neuropsychological deficits. Some studies showed that these patients had a significantly lower quality of life in comparison to the population.[5-7] In addition, many TTP patients suffer from cognitive deficits and depression.[5,8-10] Until now, few studies regarding neuropsychological consequences of TTP have been published. An increased prevalence of depression among patients with somatic diseases is known. Many studies have shown an increased prevalence of depressive symptoms among people after stroke, myocardial infarction, or certain autoimmune diseases, such as multiple sclerosis.[11-17]

In this study we investigated the prevalence of depressive symptoms and cognitive deficits in patients having survived acute TTP episodes. We undertook two surveys at an interval of 1 year to investigate possible changes over time and risk factors. The latter included the number and severity of TTP episodes, the incidence of stroke, and the occurrence of neurologic symptoms during TTP bouts, which could promote neuropsychological problems. The early identification of depressive symptoms and cognitive deficits may be important to provide the best care for the patients and to improve their quality of life.[18,19]

MATERIALS AND METHODS

The University Medical Center Mainz is a government-funded major referral center for TTP patients. Those living in the state of Rhineland-Palatinate were treated in the Department of Hematology, Oncology and Pneumology of University Medical Center Mainz and were directly included in this observational cohort study. In addition, this study includes all consecutive TTP patients for whom the major referral center Mainz was requested to give medical advice to the external hospitals. External TTP patients presented personally, usually once per year, in the Department of Hematology, Oncology and Pneumology of University Medical Center Mainz.

Patients

The study was initiated in October 2012 and since then all patients were prospectively registered, who had been treated for acute TTP and/or followed in remission at least once per year by the department of Hematology, Oncology and Pneumology at the University Medical Center of the Johannes Gutenberg University. After the approval of the ethics committee was obtained, TTP patients were interviewed once per year by means of standardized questionnaires. The first inquiry was conducted in June 2013 and the second in July 2014 including all patients aged 18 years or older with a diagnosis of TTP before starting this study and who were in remission (normal PLT count for at least 30 days after the last plasma exchange). During the course of this study, further participants with an incident diagnosis of TTP were recruited. Inclusion criteria were the clinical diagnosis of TTP, defined as a microangiopathic hemolytic anemia, thrombocytopenia ($<150 \times 10^9$/L), and severe ADAMTS13 deficiency (<10%) in acute TTP episode. ADAMTS13 activity was measured either with a commercial FRETS-assay developed by American Diagnostica, Inc. (ACTIFLUOR ADAMTS13) or since July 2014 by a fluorogenic assay using the FRETS-VWF73 substrate.[20,21]

Results of questionnaire study were related to the number and severity of acute TTP episodes and to the time interval between the last acute TTP bout and the survey. Therefore, past clinical history of TTP patients was collected retrospectively by means of medical records and prospectively since October 2012. Clinical reports of all TTP patients were examined regarding course of disease, especially neurologic symptoms during acute episodes and relapse rate. TTP-induced neurologic abnormalities were defined as presenting features if they occurred at any time during the acute episode. Neurologic signs were graded as severe, mild, or absent as reported by Vesely and colleagues.[22] The process of patients' recruitment is shown in Fig. 1. Demographic and clinical details are presented in Table 1. Additionally, healthy adults were interviewed during the second survey as a control cohort including employees and associated persons of the University Medical Center Mainz as well as students of all departments of the Johannes Gutenberg University Mainz, who did not have TTP, cancer, coronary heart disease, or neurologic disorder. These controls were about 10 years younger and the proportion of females was lower compared to the TTP patient group (Table 2). For this reason we used a linear regression model as sensitivity analysis to compare the patients and controls in both surveys for both outcome variables German questionnaire for complaints of cognitive disturbances (FLei) score and Inventory of Depressive Symptomatology, Self-Report (IDS-SR) score to adjust for age and sex. The p values do not differ compared to the p values from the univariable tests for comparing patients and controls. Furthermore, results of FLei were compared with those of healthy controls (n = 97) as well as depressive patients (n = 94) from the literature.[23] The study was approved by the Ethics Committee of "Landesärztekammer Rheinland-Pfalz" [837.265.14 (9504-F)], and all participants had given written consent to participation.

Psychometric assessment

TTP patients were twice invited to participate in the study at an interval of 10 to 12 months. At both time points, psychometric questionnaires were either sent by regular mail

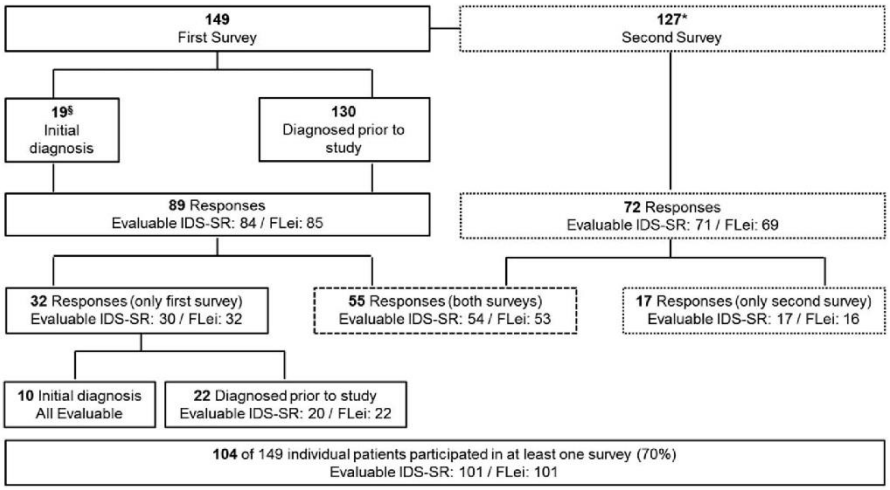

Fig. 1. Patient recruitment and response rates in two surveys of the cohort of TTP patients from Mainz. A total of 149 eligible TTP patients in remission were invited to fill out two questionnaires used in two surveys each. A total of 130 of the 149 patients had been diagnosed with TTP before starting this study whereas 19 patients had their initial diagnosis of TTP during this study. *Between the first and second surveys two of the 130 invited patients died from a cause unrelated to TTP and one patient was lost to follow-up. §The 19 patients newly diagnosed during the first survey were not yet subjected to the second survey. Accordingly, 127 TTP patients were sent the questionnaires in the second survey after approximately 1 year. Response rates and number of evaluable questionnaires are indicated.

TABLE 1. Characteristics of the responding and evaluable TTP patients in both surveys and of the healthy controls*

Characteristics	TTP patients first survey	TTP patients second survey	Healthy controls
Number	85	71	52
Sex			
Female	68 (80)	60 (85)	31 (61)
Male	17 (20)	11 (15)	20 (39)
Age (years)	46 (18-84)	45 (22-85)	35 (21-87)
Psychotherapy			
Before TTP diagnosis	9 (11)	NA	11 (21)
After TTP diagnosis	17 (20)	NA	Not applicable
At the time of questioning	6 (7)	6 (8)	3 (6)
Psychopharmacotherapy	NA*	10 (14)	NA*

* Data are reported as number (%) or median (range).
NA = not available.

to the patients' home or given directly to patients before discharge from hospital. We used standardized questionnaires to evaluate depression (IDS-SR)[24,25] and mental performance comprising executive function, memory, and attention (FLei, "Fragebogen zur subjektiven Einschätzung der geistigen Leistungsfähigkeit").[23]

IDS-SR

Depression was assessed by the German version of the IDS-SR. The IDS-SR consists of 30 items assessing the presence and severity of symptoms and detecting changes of symptoms during acute phase treatment.[25] Each of the 30 items is rated by a score from 0 to 3 with increasing severity represented as a higher score.[24] The total score ranges from 0 to 84 and is interpreted as follows: 0-13: no depression; 14-25: mild depression; 26-38: moderate depression; 39-48: severe depression; 49-84: very severe depression. The IDS-SR is one of the few validated questionnaires that deliver comparable results when used for self-diagnosis and as an assessment tool for the physician.

TABLE 2. Results of mental performance impairment by means of the FLei score in TTP patients*

	TTP patients				
Test domain	First survey	Second survey	Healthy controls	Healthy controls[23]	Depressive controls[23]
Number	85	69	52	97	94
Total FLei score	38.8 (±26.2)[a,b]	37.5 (±26.2)[a,c]	16.7 (±10.8)	29.1 (±18.7)	56.5 (±23.1)
Subscores memory	13.9 (±9.1)[a,c]	13.8 (±9.1)[a,c]	7.2 (±4.3)	10.8 (±6.6)	18.3 (±7.9)
Attention	13.5 (±9.6)[a,b]	12.9 (±9.8)[a,c]	4.8 (±3.6)	9.7 (±6.5)	19.2 (±8.4)
Executive function	11.5 (±8.5)[a,c]	10.8 (±8.5)[a,d]	4.7 (±4.1)	8.7 (±6.7)	19.0 (±8.5)

* Total score points and subscores for memory, attention, and executive function [mean (±SD)] in TTP patients, in comparison to our healthy controls, as well as to healthy and depressive controls from the literature.[23] Mental performance was significantly worse for TTP patients in each survey in comparison to both healthy cohorts ([b]$p < 0.001$, [c]$p < 0.01$, and [d]$p < 0.05$). TTP patients performed significantly ([a]$p < 0.001$) better than the depressive controls from the literature.

This test is well suited to detect symptoms of depression and any changes occurring during the course of the depression. A comparison of the IDS-SR with other standardized questionnaires shows good agreement for grading the severity of depression.[25] Comparing the widely used Beck Depression Inventory to the IDS-SR suggests that a clinically relevant depression can be confidently diagnosed for depression score of more than 25 on the IDS-SR.[24,25]

FLei

Cognitive deficits were assessed by the FLei, a self-report measure with 30 items covering the subscores of deficient attention, memory, and executive functions, with 10 items each. All items are rated on a 5-point Likert scale (0 = at no time; 4 = very frequent). Accordingly, total score for all 30 items ranges between 0 and 120 points. The internal consistencies of the three subscores (Cronbach's alpha and split-half reliability) are all more than 0.87.[23] Healthy controls reported in the literature showed a mean of 29.1 (standard deviation [SD] ± 18.7) whereas controls with major depression (ICD.10) had a mean of 56.5 (SD ± 23.1; Table 2).[23]

Covariates

Age, sex, and clinical characteristics including time interval since the last acute TTP episode, total number of TTP bouts, presence or absence of neurologic symptoms during the acute TTP attacks, and occurrence of stroke were obtained from medical records. Information concerning psychotherapy and antidepressive medication was collected by questioning the participants.

Statistical analysis

Statistical analyses were performed using computer software (SPSS, Version 22.0, IBM GmbH). Descriptive statistics included frequency, mean, SD, median, interquartile range (IQR), minimum, and maximum. Differences between two groups were tested using t test for normally distributed data and the nonparametric Mann-Whitney U test for nonnormally distributed outcomes, as well as the Kruskal-Wallis test for comparing patients with one versus two versus three or more TTP episodes. As the patient and control groups differ in age and sex distribution, a linear regression model was additionally used for comparing the FLei score between the patient and the control group to adjust for age and sex. For comparing changes in FLei score between patients, who completed the first and second surveys, a dependent t test was used as well as for the IDS-SR score the dependent Wilcoxon test. Spearman's rank correlation coefficients (r_s) were calculated to estimate the relationships between depressive symptoms or cognitive deficits, respectively, and the number of TTP episodes and to estimate the relationship between depressive symptoms and cognitive deficits. p values less than 0.05 were considered as significant. All p values should be treated with caution as no adjustment for multiple testing was performed.

RESULTS

Study population

From June 2013 until November 2014, a total of 148 patients with a clinical diagnosis of an acquired TTP, one with a hereditary TTP, and 52 healthy controls were asked to participate. A total of 130 of the 149 patients had been diagnosed with TTP before starting this study and were in remission at the time of investigation. Nineteen patients had their initial diagnosis of TTP during this study; they were evaluated in the first survey after achieving remission (Fig. 1). Between the first and second surveys, two of the 130 participants having been diagnosed with TTP before starting the study died from a cause unrelated to TTP and one patient was lost to follow-up. The 19 patients newly diagnosed during and after the first survey were not yet subjected to the second survey. Accordingly, 127 TTP patients were sent the questionnaires in the second survey, approximately 1 year after the first inquiry.

We received 89 responses in the first survey, 85 of which were evaluable for FLei and 84 for IDS-SR (Fig. 1).

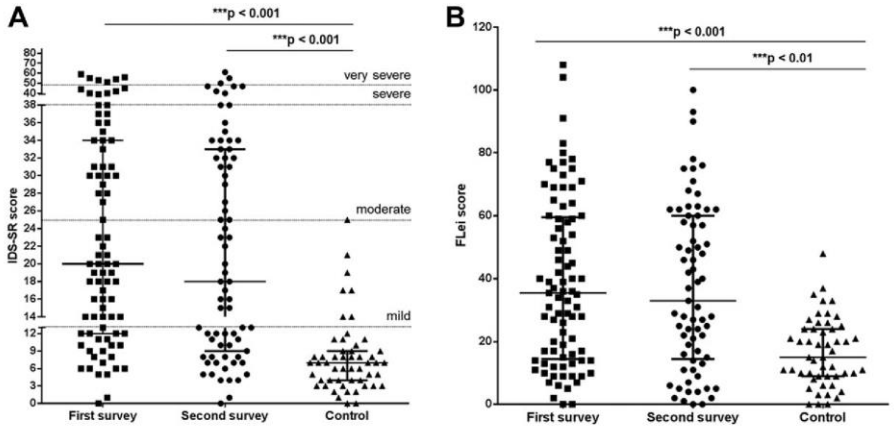

Fig. 2. Results of depression (IDS-SR) and cognitive impairment (FLei) of TTP patients in the two surveys and of the healthy controls. (A) IDS-SR score for the TTP patients at the first (n = 84) and at the second surveys (n = 71) compared to healthy controls (n = 52). For the first survey the median evaluated score points were 20 (IQR, 12-34), for the second survey 18 (IQR, 9-33), and for the healthy controls 7 (IQR, 4-9). The prevalence of depressive symptoms in TTP patients was significantly higher in both surveys than in controls ($p < 0.001$ each). (B) FLei score for the TTP patients at the first (n = 85) and at the second surveys (n = 69) in comparison to healthy controls (n = 51). For the first survey the median evaluated score points were 35.5 (IQR, 15-60), for the second survey 33 (IQR, 15-60), and for the healthy controls 15 (IQR, 9-24). Mental performance was significantly worse for TTP patients in both surveys in comparison to the healthy controls ($p < 0.001$ and $p < 0.01$, respectively).

Seventy-two responses were obtained in the second survey, 69 were evaluable for FLei and 71 for IDS-SR. Overall, responses of 101 individual TTP patients were evaluable for IDS-SR and FLei, respectively, and 54 IDS-SR and 53 FLei questionnaires were evaluable for both the first and the second surveys. Thirty-two TTP patients participated only in the first survey and 17 only in the second survey (Fig. 1). Results of impairment of cognitive performance (FLei) of the TTP patients were compared with FLei scores of 97 healthy controls as well as 94 patients with diagnosed depression (ICD-10) from the literature[23] (Table 2).

Patient characteristics

The characteristics of the TTP patients and the healthy controls are shown in Table 1. Participants were predominantly female (first survey 80% females, second survey 85% females) with a mean age of approximately 45 years spanning a wide range from 18 to 85 years. Fifty-five percent of the TTP patients had children, 31% were single, 58% were married, and 11% were divorced. Fifty-one percent of TTP patients stated that they have distress. Forty-eight percent of the TTP cohort were overweight (body mass index > 25). Medical records of 60 of the 104 individual TTP patients were completely evaluable regarding neurologic symptoms during acute episodes and relapse rate.

IDS-SR results

In 2013, a total of 61 (72.6%) of 84 TTP patients were scored to have current depressive symptoms by IDS-SR (score points ≥14). The median evaluated score points were 20 (IQR, 12-34), ranging from 0 to 59 (Fig. 2A). Twenty-three (27.4%) patients had no, 27 (32.1%) had mild, 22 (26.2%) had moderate, six (7.1%) had severe, and six (7.1%) had very severe depression.

At first survey, 20% of the patients stated having received psychotherapy after being diagnosed with TTP, 11% had received psychotherapy before the diagnosis of TTP, and 7% were receiving psychotherapy at the time of answering the questionnaire (Table 1). In 2014, 42 (59.2%) of 71 TTP patients were scored to have current depressive symptoms by IDS-SR (score points ≥14). The median total score was 18 (IQR, 9-33), ranging from 0 to 61 score points (Fig. 2A). Regarding severity of depression, 29 (40.8%) patients had no, 15 (21.1%) had mild, 19 (26.8%) had moderate, five (7.0%) had severe, and three (4.3%) had very severe depression.

At the time of questioning six (8%) TTP patients received psychotherapy and 10 (14%) were treated with antidepressants (Table 1). Seven of 52 (13.5%) healthy controls had score points suggesting depressive symptoms (score points ≥ 14; Fig. 2A). None of these 52 had a clinically relevant depression (>25). This prevalence of mild

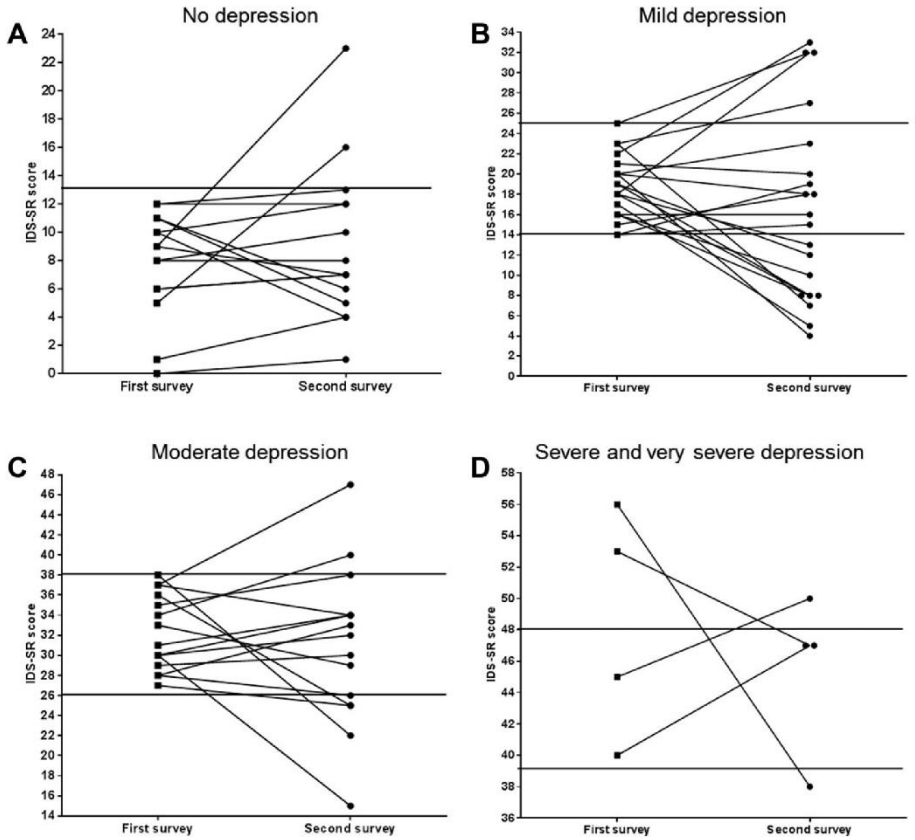

Fig. 3. Course of depression in 54 TTP patients with evaluable IDS-SR scores in both first and second surveys. (A) Fifteen TTP patients with no depression (score points, 0-13) in the first survey. (B) Twenty TTP patients with mild depression (score points, 14-25) in the first survey. (C) Fifteen TTP patients with moderate depression (score points, 26-38) in the first survey. (D) Two TTP patients with severe (score points, 39-48) and two with very severe depression (score points, 49-84) in the first survey.

depression in controls complies with the 12-month prevalence in Germany (9.3% total, 6.1% in men, and 12.4% in women) as well as with the expected prevalence in the United States (6%).[9,26]

The prevalence of depression in TTP patients was significantly higher in both surveys than in controls ($p < 0.001$). No differences concerning prevalence and severity of depression between the TTP patients in both surveys was detected.

Fifty-five TTP patients responded both in the first and in the second surveys and 54 of them were evaluable for IDS-SR twice. The severity of depression for most of these patients was similar in both surveys (Fig. 3). Most of the 19 TTP patients with clinically relevant depressive symptoms (IDS-SR score > 25) in the first survey had no improvement; only four cases with clinically relevant, moderate, depressive symptoms improved (Figs. 3C and 3D). On the other hand, TTP patients with no or mild depressive symptoms did not develop clinically relevant depressive symptoms in most instances; only four of 35 TTP patients with no or mild symptoms developed a moderate depression (Figs. 3A and 3B).

FLei results

Eighty-five TTP patients in the first and 69 in the second surveys were evaluable for their mental performance by

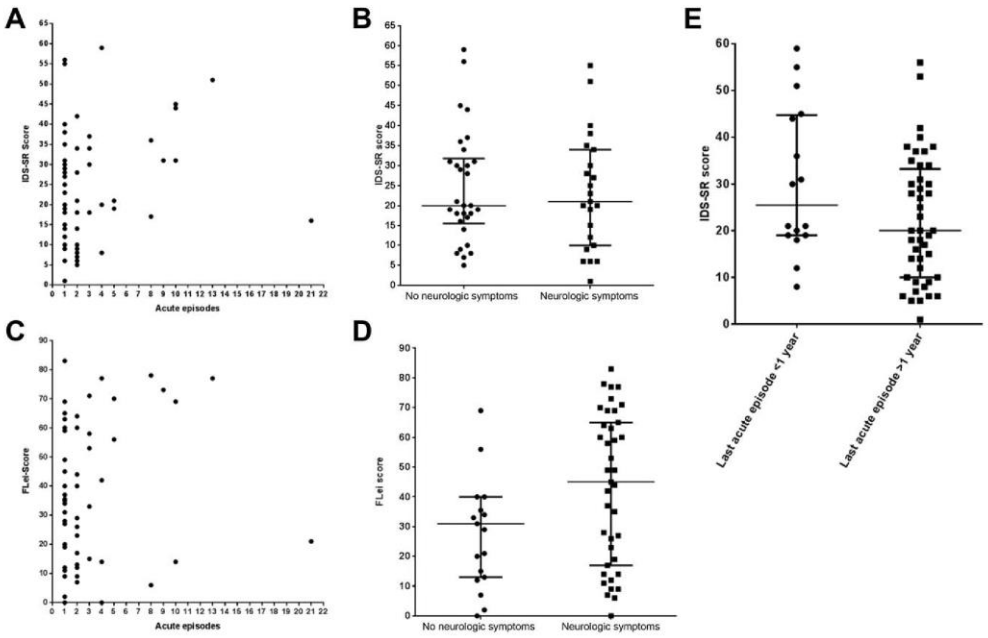

Fig. 4. Association of depressive symptoms and cognitive deficits with number and severity of acute TTP attacks. (A) Correlation between IDS-SR score and the number of acute TTP episodes (median, 1) in the first survey (r_s = 0.157, NS) for 57 evaluable TTP patients. Comparison of TTP patients with one versus two versus three and more acute episodes (Kruskal-Wallis test, p = 0.011). (B) In the first survey 30 TTP patients (IDS-SR median, 20 [IQR, 16-32]) had no and 23 TTP patients (IDS-SR median, 21 [IQR, 10-34]) had neurologic symptoms in the last acute TTP episode. There was no difference between severity of depression in patients with or without neurologic symptoms (Mann-Whitney U test, p = 0.781, NS). (C) Correlation between cognitive deficits (FLei score) and the number of acute TTP episodes (median, 1) in the first survey (r_s = 0.115, NS) for 58 evaluable TTP patients. Comparison of TTP patients with one versus two versus three and more acute episodes (Kruskal-Wallis test, p = 0.078). (D) Cognitive deficits and presence or absence of neurologic symptoms during any acute TTP episode in the first survey. Median FLei score in 17 TTP patients without neurologic symptoms was 31 (IQR, 13-40), and median FLei score in 39 patients with neurologic symptoms was 45 (IQR, 15-65; Mann-Whitney U test, p = 0.193, NS). (E) IDS-SR score for the TTP patients with an acute TTP episode within the past year (n = 16) in comparison to those with the last TTP attack more than 1 year ago (n = 44; Mann-Whitney U test, p = 0.063, NS).

FLei (Fig. 2B). The total score points in both surveys were normally distributed and showed a mean of 38.8 (SD ± 26.2) in the first and a mean of 37.5 (SD ± 26.2) in the second survey, ranging from 0 to 108 (Fig. 2B, Table 2). Mental performance, as well as the three subscores, were significantly worse for TTP patients in both surveys in comparison to both healthy cohorts (Table 2). Nevertheless, TTP patients performed significantly ($p < 0.001$) better than depressive patients from the literature (Table 2). The comparison of the FLei results for the 53 TTP patients who were evaluable in both surveys showed nearly identical mental performance scores (data not shown).

Association of depressive symptoms and cognitive deficits with number and severity of acute TTP attacks

The exact course of disease could be ascertained for 60 of 104 (58%) patients for whom all clinical records were available. These patients were selected to assess the relation of IDS-SR and FLei to characteristics of TTP. We evaluated the time since the initial TTP diagnosis, the number of TTP bouts, the time span since the last acute episode of TTP, and the symptoms during acute disease. We found no positive correlation of presence or severity of depression (first survey r_s = 0.157; second survey r_s = 0.023) or cognitive deficits (first survey r_s = 0.115; second survey

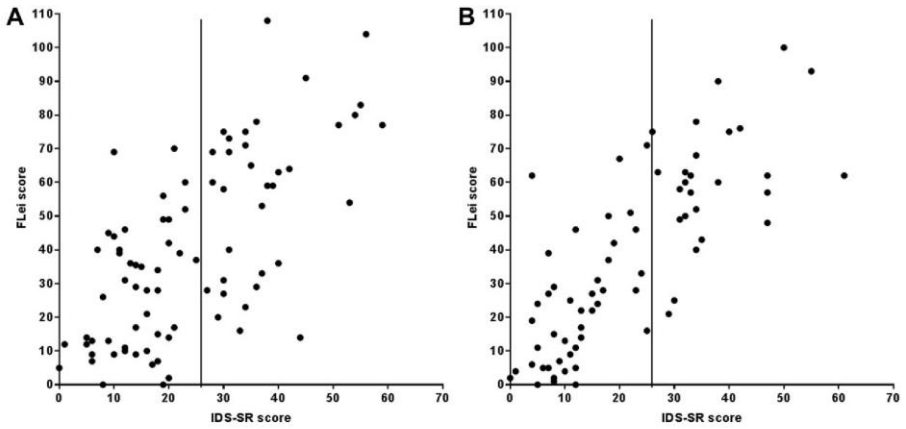

Fig. 5. Correlation of FLei score with IDS-SR score. (A) Correlation of FLei and IDS-SR in the first survey of 84 TTP patients ($r_s = 0.643$, $p < 0.001$). (B) Correlation of FLei and IDS-SR in the second survey of 69 TTP patients ($r_s = 0.779$, $p < 0.001$). Clinically relevant depression (IDS-SR score > 25) is indicated by a vertical line.

$r_s = 0.092$) and the number of survived acute episodes (Figs. 4A and C). Solely in the first survey, TTP patients with one acute bout were less depressive than patients with two and more acute bouts (Kuskal-Wallis test $p = 0.011$). Similarly, IDS-SR (55 patients first survey, $p = 0.466$, Fig. 4B; 48 patients second survey, $p = 0.367$, not shown) and FLei (56 patients first survey, $p = 0.193$, Fig. 4D; 47 patients second survey, $p = 0.793$, not shown) were not significantly different in patients having suffered from neurologic symptoms compared to those without neurologic manifestation. Nine patients had suffered a stroke in their clinical history. Six of them had an IDS-SR score of more than 25 and a mean value of 72 (range, 36-93) in the FLei score. Furthermore, we investigated the correlation between depression (Fig. 4E) and cognitive deficits (not shown), respectively, and the time interval since last acute episode until survey. Sixteen (27%) TTP patients had their last acute episode within the past year and 44 (73%) more than 1 year before the inquiry. A trend was detectable that patients with a most recent TTP episode within the past year had higher depression scores (median, 26; IQR, 19-45) than patients with no bout in the past year (median, 20; IQR, 10-33; $p = 0.063$; NS). In summary, no significant correlation between severity of depression or magnitude of cognitive deficits and the frequency or the severity of acute TTP episodes, respectively, was detectable.

Correlation of depression and impaired cognitive performance

Impaired mental performance correlated strongly with the severity of depression (Fig. 5). Spearman's rank correlation coefficient (r_s) for 84 TTP patients in the first survey was 0.643 ($p < 0.001$; Fig. 5A) and for 69 participants in the second survey 0.779 ($p < 0.001$; Fig. 5B). Overall, correlation of FLei and IDS-SR from 101 individual TTP patients revealed an r_s of 0.696 ($p < 0.001$). TTP patients without clinically relevant depression (IDS-SR ≤ 25) had FLei scores (mean first survey, 26.4; mean second survey, 23.2) not different to healthy controls (mean, 29.1; Fig. 5). TTP patients with clinically relevant depression (IDS-SR score > 25) had FLei scores (mean first survey, 56.6; mean second survey, 61.0) not different from depressive controls (mean, 56.5; Fig. 5). TTP patients with severe and very severe depression performed slightly, not significantly, worse in the FLei score than depressive patients from the literature (Table 2).

DISCUSSION

Treatment of TTP currently focuses on survival of acute episodes, the mortality still being approximately 10% to 20%.[4] Possible long-term consequences of TTP have not been given major attention until recently. The prevalence of depression in survivors of TTP in our surveys exceeds the 12-month prevalence in the German general population (9.3%), in women (12.4%) and in men (6.1%).[26] Deford and colleagues[9] reported a point prevalence of 19% for major depression, which is significantly higher than in the local population and similar to the prevalence of severe and very severe depression together (14 and 11%, respectively, in the two surveys of our patients). Focusing on all patients with clinically relevant depression (IDS-SR > 25) we observed 40% in the first and 38% in the

second surveys. These results are consistent with the report of Han and coworkers[27] who reported a point prevalence of 46% for clinically relevant depression investigating 35 TTP patients assessed by Beck Depression Inventory. Other severe diseases, both acute and chronic, are associated with an increased rate of depression. Autoimmune diseases such as multiple sclerosis,[16,28] which progress with relapses, or systemic lupus erythematodes are associated with a high prevalence of depression.[27] Depression also frequently occurs after a myocardial infarction[29] or stroke.[13,30,31] Depressive symptoms can contribute to impaired quality of life and herald an unfavorable prognosis of the original disease including an increased morbidity and mortality.[9,18,19] Lewis and coworkers[6] investigated the quality of life in 118 and Cataland and coworkers[5] in 27 TTP patients by using the SF-36 questionnaire. These patients exhibited a significant decrease in quality of life compared to the general population. To assess the patients' everyday impairment, we decided to use the subjective FLei questionnaire, which encompasses tests for memory, attention, and executive function. Our TTP patients reported a significant reduction of their cognitive performance compared with healthy controls; however, they performed significantly better than depressive controls reported from the literature.[23] This is in line with findings of Kennedy and colleagues[8] as well as Cataland and colleagues[5] who described impaired cognitive performance, albeit both groups used clinical evaluation methods instead of self-reports. The significant correlation between severity of depression and the degree to which cognitive performance was reduced raises the question whether impaired cognitive performance is a direct consequence of depression rather than a result of prior cerebral microvascular thrombosis and ischemia. This hypothesis is supported by the fact that TTP patients who did not suffer from clinically relevant depression performed at least equally well in FLei as healthy controls, independently of severity and number of prior acute TTP episodes. TTP patients with severe or very severe depression perform identically to depressive cohorts reported in the literature.[23] An association of depression and relevant cognitive problems is described in the literature.[32,33] Kennedy and colleagues[8] suggested that the cognitive impairment might result from the diffuse cerebral microvascular thrombosis. On the other hand, Han and coworkers[27] found no relationship between depression and cognitive performance in TTP survivors; however, they compared the Beck Depression Inventory, a questionnaire for self-evaluation of depression, with a psychiatric assessment of cognitive performance. Thus, the results of Han and colleagues[27] support our hypothesis that self-estimated cognitive impairment may primarily be ascribed to depression. The degree to which reduction in cognitive performance is caused by microvascular thrombosis during acute bouts of TTP and to which extent impaired cognitive performance is a direct result of depression is unclear and needs further prospective investigation.

Our study has limitations: first, our measure of cognitive performance relied on self-report in contrast to the report by Kennedy and coworkers.[8] Thus, we cannot preclude that cognitive alteration was affected by a general negative self-evaluation that is a leading sign of depression. On the other hand, the questionnaire on cognitive performance is well validated. A further limitation is the comparison of TTP patients with depressive patients only from the literature. The severity of depression of these patients is unknown. Thereby possible confounders could not be considered, for example, chronicity or therapy characteristics. For our observational study on TTP survivors we have no sex- and gender-matched control subjects but rather a general somewhat younger healthy control cohort. However, we used questionnaires that were widely validated in a large cohort and they are applicable without a control group. Second, we did not know why only approximately 60% of the TTP survivors participated in the self-evaluation study. It may be possible that symptomatic patients were more prone to answer the survey compared to asymptomatic patients. Finally, detailed clinical information on severity of TTP bouts was available in only approximately 60% of participating patients. Therefore, prospective assessment of all consecutive patients is initiated.

In conclusion, our observational study illustrates that the prevalence of depression and cognitive deficits was significantly higher in our cohort of 104 individual TTP survivors than in the German population. Despite the high prevalence of depression, only very few (7%) patients were undergoing psychological or psychotherapeutic treatment. We did not detect a significant correlation between severity of depression or cognitive deficits and the frequency or severity of acute TTP episodes. Nevertheless, we demonstrate a highly significant correlation between severity of depression and the degree to which cognitive performance was reduced. Whether or not depression itself directly causes reduced cognitive performance, clinicians should carefully assess depressive and neurocognitive symptoms in TTP survivors and initiate adequate treatment to improve their quality of life.

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manuscript, approval; GH—revision of the manuscript, approval; MB—psychosomatic advice, revision of the manuscript, approval; KL—revision of the manuscript, approval; BL—data compilation, revision of the manuscript, writing of the manuscript, approval; and IS—study supervision, revision of the manuscript, writing of the manuscript, approval.

CONFLICT OF INTEREST

IS a member of the Data Safety Monitoring Board in the BAX 930 study (investigating recombinant ADAMTS13 infusion in hereditary TTP). She received travel and accommodation support for participating at scientific congresses or meetings from Bayer and NovoNordisk. BL was chairman of the Data Safety Monitoring Board in the BAX 930 study (investigating recombinant ADAMTS13 infusion in hereditary TTP). He holds a patent on ADAMTS13 and received travel and accommodation support for participating at scientific congresses or meetings from Baxalta, Siemens, Alexion, and Ablynx and speaker's fee from Siemens. GH has received speaker's fee from Servier0020Deutschland GmbH. She reports no conflict of interest with this publication.

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7.3. Originalarbeit III.

Falter T, Herold S, Weyer-Elberich V, Scheiner C, Schmitt V, von Auer C, Messmer X, Wild P, Lackner KJ, Lämmle B, Scharrer I. Relapse Rate in Survivors of Acute Autoimmune Thrombotic Thrombocytopenic Purpura Treated with or without Rituximab. Thromb Haemost. 2018 Oct;118(10):1743-1751.

Relapse Rate in Survivors of Acute Autoimmune Thrombotic Thrombocytopenic Purpura Treated with or without Rituximab

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Thromb Haemost 2018;118:1743–1751.

Abstract

Background Autoimmune thrombotic thrombocytopenic purpura (iTTP) is caused by autoantibody-mediated severe a disintegrin and metalloprotease with thrombospondin type 1 repeats, member 13 (ADAMTS13) deficiency leading to micro-angiopathic haemolytic anaemia (MAHA) and thrombocytopenia with organ damage. Patients survive with plasma exchange (PEX), fresh frozen plasma replacement and corticosteroid treatment. Anti-CD20 monoclonal antibody rituximab is increasingly used in patients resistant to conventional PEX or relapsing after an acute bout.

Objective This retrospective observational study focused on the relapse rate and possible influencing factors including treatment with rituximab first introduced in 2003.

Patients and Methods Seventy patients treated between January 2003 and November 2014 were evaluated. Number, duration, clinical manifestations, laboratory data and treatment of acute episodes were documented. Diagnostic criteria of acute iTTP were thrombocytopenia, MAHA, increased lactate dehydrogenase and severe ADAMTS13 deficiency.

Results Fifty-four female and 16 male patients had a total of 224 acute episodes over a median observation period of 8.3 years. The relapse rate was 2.6% per month, for women 2.4% and for men 3.5% per month. Since 2003, 17 patients with a first iTTP episode were treated with rituximab, whereas 28 were not. There was a trend towards lower relapse rates after rituximab treatment over the ensuing years. However, this was statistically not significant.

Keywords
- ADAMS/ADAMTS13
- thrombotic thrombocytopenic purpura (TTP/HUS)
- clinical studies
- autoantibodies

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Conclusion This analysis does not show a significant reduction of acute iTTP relapses by rituximab given during an acute bout. Initial episodes are characterized by more severe clinical signs compared with the less severe relapses. Furthermore, men suffer significantly more frequent and considerably more serious acute relapses.

Introduction

Autoimmune thrombotic thrombocytopenic purpura (iTTP) is an acute, life-threatening disorder, and survivors are at risk of disease relapse. Acute episodes are characterized by consumptive thrombocytopenia, micro-angiopathic haemolytic anaemia (MAHA) and spontaneous von Willebrand factor (VWF)-induced platelet clumping. A severe a disintegrin and metalloprotease with thrombospondin type 1 repeats, member 13 (ADAMTS13) deficiency (< 10%) due to autoantibodies against ADAMTS13 is associated with acute episodes. ADAMTS13 regulates the size of the newly synthesized and secreted ultra-large (UL) VWF multimers, and cleaves these into less haemostatically active forms.[1,2] Symptoms of iTTP are highly variable. Many patients suffer from neurological symptoms such as confusion, headache, paresis, aphasia and coma. Thrombocytopenia can result in petechiae, epistaxis and gingival bleeding. Further symptoms are abdominal pain, nausea, fatigue, proteinuria and cardiac complications.[3] Patients surviving an acute iTTP bout and showing normalization of the laboratory parameters are often considered to be cured; however, recurrences of acute iTTP attacks are common.[2,4]

Since 1991, plasma exchange (PEX) and corticosteroids are the primary standard of care in iTTP.[5,6] However, there is a proportion of iTTP patients having refractory acute episodes, exacerbations or a high tendency to relapse. Since 2002, rituximab, an anti-CD20 monoclonal antibody, was used in the treatment of iTTP and has become an international standard of therapy.[7] PEX removes ADAMTS13 autoantibodies as well as UL VWF multimers and fresh frozen plasma (FFP) replacement supplies ADAMTS13. Efficacy of PEX reducing mortality in acute episodes from 90 to 20 to 30% is indisputable[3,4,6]; nevertheless, PEX and corticosteroids are not as efficient in suppressing autoantibodies as rituximab. Rituximab suppresses the disease-associated ADAMTS13 inhibitor production by depleting B lymphocytes.[8] Despite widespread application in iTTP, rituximab is still an off-label use.

In the current retrospective observational study, we investigated iTTP patients focusing on the relapse rate and possible influencing factors including treatment with rituximab.

Patients and Methods

In this systematic retrospective study, we analysed all iTTP patients referred to the University Medical Center (UMC) Mainz from January 2003 to November 2014. Subjects had an acute iTTP bout or were consulting our institution having survived an earlier acute iTTP episode. Inclusion criteria were the clinical diagnosis of iTTP, defined as thrombocytopenia (< 150,000/µL), MAHA, increased lactate dehydrogenase (LDH; > 1.5× upper limit of normal values) with or without ischaemic organ damage. Since 2003, severe ADAMTS13 deficiency (< 10%) caused by an ADAMTS13 autoantibody during an acute bout is an additional diagnostic requirement for iTTP.

Acute iTTP episodes were treated according to a standard procedure of the UMC Mainz and in accordance with the international guidelines. All iTTP patients received PEX using FFP or Octaplas SD (Octapharma, Vienna, Austria) daily from admission until platelet count of > 150,000/µL was reached for longer than 48 hours. From the first day of acute iTTP corticosteroids, usually prednisolone, 1 to 2 mg/kg body weight, were given daily.

In off-label use, rituximab was administered for the first time in 2003. In this iTTP cohort, rituximab (MabThera; Roche, Grenzach-Wyhlen, Germany) was only used in acute iTTP bouts (1–4 weekly infusions of 375 mg/m^2 each). In most cases, rituximab was administered to patients with thrombocytopenia persisting under daily PEX for ≥5 days. A second indication for rituximab treatment was in relapsing iTTP patients without severe organ manifestation where rituximab was given to avoid PEX.

Complete remission was defined as full resolution of the clinical manifestations, especially neurological symptoms, with normalized platelet count for more than 30 consecutive days after the last PEX.[9]

This retrospective study was approved by German law (Landeskrankenhausgesetz §36 and §37) in accordance with the Declaration of Helsinki and by the Ethics Committee of "Landesärztekammer Rheinland-Pfalz" [837.506.15 (10274)].

Assays

If plasma ADAMTS13 activity was measured between 1996 and 2002, the VWF multimer degradation method (immunoblotting) was used.[1,4] Since January 2003, ADAMTS13 activity was examined by the residual ristocetin co-factor-based method.[10] Since April 2010, it was examined by the fluorescence resonance energy transfer system (FRETS-VWF73) method[11] modified according to Kremer Hovinga et al.[12] ADAMTS13 activity was expressed as percentage relative to that of pooled normal plasma. The normal range of ADAMTS13 activity in the ristocetin co-factor-based method was defined as 52 to 134% in 80 healthy controls with a detection limit of 6.25%. The normal range for the FRETS-VWF73 assay in healthy donors is > 50% with a limit of detection of 1%.[4,12]

ADAMTS13 inhibitor was detected by incubating a mixture of heat-inactivated patient plasma with pooled normal plasma (1:1; v:v) for 60 minutes at 37°C and then measuring the ADAMTS13 activity by the FRETS-VWF73 assay.[12] Inhibition of 50% of normal plasma ADAMTS13 activity by undiluted patient

plasma was defined as 1 Bethesda unit (BU) per mL. An ADAMTS13 inhibitor was diagnosed when ≥0.5 BU/mL were found.

Variables/Covariates

The following data of iTTP patients were collected: date of birth, sex, number of biological children, body height and weight, calculated body mass index, smoking status and possible co-morbidities.

Data concerning the acute iTTP bouts of each patient was collected such as number of acute bouts, clinical symptoms, beginning and end of the respective bout (admission into and discharge from a hospital), calculated time of an acute episode, calculated age at a bout and calculated whole observation time. Therapeutic procedures (PEX, immune adsorption or splenectomy) and medication (corticosteroids, rituximab, other) given during an acute episode were documented.

Furthermore, we collected laboratory data such as platelet count, LDH, haemoglobin, presence of schistocytes, ADAMTS13 activity and ADAMTS13 inhibitors.

Clinical Severity Score

To classify the severity of an iTTP bout, we established a score based on clinical and laboratory data (►**Table 1**). It divides all iTTP bouts into five categories. Category 0 defines acute episodes characterized by all laboratory abnormalities listed in ►**Table 1** only without manifest clinical signs, usually encountered in known iTTP patients during outpatient visits. Category 1 involves mild clinical symptoms and all the laboratory abnormalities as in category 0. Category 2 and category 3 include more severe clinical signs and symptoms. Acute iTTP episodes with fatal outcome were grouped in category 4 (►**Table 1**).

Statistical Analysis

We organized all data in a SPSS file pseudonymizing patient names according to the guidelines of the ethics committee.

For descriptive analysis, median and interquartile range (IQR), as well as minimum and maximum were calculated for continuous variables. In addition, absolute and relative frequencies were computed for categorical variables and visualized via bar charts. For interpreting the recurrent events, a graphic is created with one line for each patient on the *y*-axis with dots for each recurring event with observation time on the *x*-axis. Kaplan–Meier estimates were used to describe the relapse-free survival times for patients who did or did not receive rituximab. The log-rank test was used to compare the curves. Additionally, a Cox proportional hazard regression model was used to evaluate the effect of explorative variables on relapse-free survival.

To model the number of events for each patient and specifically to estimate the relapse rate in different subgroups, a Poisson model with the log-transformed observation time incorporated as offset variable was performed.

For confirmatory analysis to estimate the rate ratio for patients treated with or without rituximab, adjusting for the variables sex and age, an Anderson–Gill model for the recurrent events was performed. The Poisson model and the Anderson–Gill model take into account the dependency between all iTTP episodes in one individual patient. The significance level was chosen to be 0.05. We present the rate ratio with its 95% confidence interval (CI) and its *p*-value. All statistical analyses were performed using SPSS version 22.0 (IBM GmbH, Ehningen, Germany) or the statistical program R version 3.4.1.

Table 1 Clinical severity score

0 = laboratory abnormalities only	All four laboratory abnormalities listed must be present • thrombocytopenia (< 150,000/μL) • increased LDH (> 1.5× upper limit of normal) • decreased haemoglobin (< 12 g/dL in females, < 14 g/dL in males) • presence of schistocytes
1 = mild	Laboratory abnormalities plus at least one of the following clinical manifestations • haematoma, petechiae, ecchymoses • cephalgia, vertigo, nausea • fatigue, drowsiness, weakness • (sub-)febrile temperatures, shivering • pain, especially abdominal pain
2 = moderate	Laboratory abnormalities plus at least one of the following clinical manifestations • micro- or macro-haematuria • icterus • tachycardia, dyspnoea, • reversible dys- or paresthesia, visual field defects • impaired consciousness (somnolence, stupor), disorientation
3 = severe	Laboratory abnormalities plus at least one of the following clinical manifestations • stroke with aphasia and/or paresia and/or apraxia and/or ataxia • acute myocardial infarction • acute renal failure, multi-organ failure • coma, seizure • in case of pregnancy abortion or stillbirth
4 = lethal	iTTP episode with fatal outcome

Abbreviations: iTTP, autoimmune thrombotic thrombocytopenic purpura; LDH, lactate dehydrogenase.

Results

Recruitment and Characteristics of 70 iTTP Patients

Since January 2003 until November 2014, a total of 88 patients were seen at the UMC Mainz for suspected iTTP. One young woman died during transfer to the UMC in her first acute iTTP episode (ascertained by an autopsy). Seventy of them demonstrated an ADAMTS13 deficiency and an ADAMTS13 inhibiting autoantibody (►**Fig. 1**). Sixty-five of the 70 TTP patients had a documented severe ADAMTS13 deficiency (< 10%) during their acute TTP episode and a detectable ADAMTS13 inhibitor. The other five iTTP patients consulted the UMC Mainz after their first acute episode and suffered from further relapses

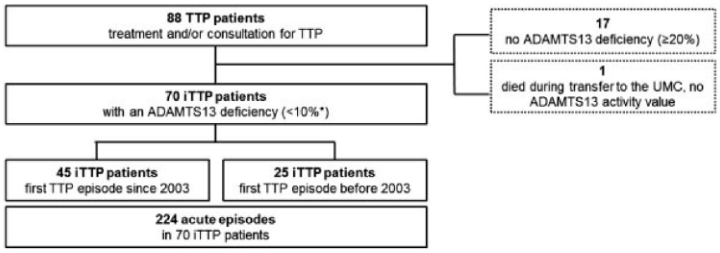

Fig. 1 Patients' recruitment. *Five autoimmune thrombotic thrombocytopenic purpura (iTTP) patients consulted the UMC after having survived their first acute episode. At the time of consultation they showed a disintegrin and metalloprotease with thrombospondin type 1 repeats, member 13 (ADAMTS13) activities of 16, 19, 15, 17 and 18%, with/ without weak ADAMTS13 inhibitors. All other iTTP patients had documented severe ADAMTS13 deficiency (< 10%) during acute episode.

until 2014. When consulting in remission, they showed ADAMTS13 activities of 16, 19, 15, 17 and 18% with or without weak ADAMTS13 inhibitor titers. The initially treating physicians of those five patients did not have the technical possibilities to measure ADAMTS13 activity or an ADAMTS13 inhibitor on site. Forty-five patients had their first acute iTTP episode during the recruiting period, and 25 iTTP patients had been diagnosed before January 2003. We started our retrospective analysis in 2003, because at that time rituximab was administered for the first time at our institution.

The characteristics of the 70 iTTP patients are shown in ►**Table 2**. The iTTP cohort consisted predominantly of females (77%) and all patients were Caucasian. Overall, 70 iTTP patients suffered from 224 acute episodes over an observation time of 8.3 years (median; range, 0.4–31.9 years, IQR, 4.3–14.3 years). The median age at diagnosis is 33 years, ranging from 12 to 64 years (IQR, 26–49 years) (►**Table 2**).

Table 2 Patients' characteristics

Characteristics	No.	%
Total number of iTTP patients	70	
Gender		
Female	54	77.1
Male	16	22.9
Ethnicity (white Caucasian)	70	100
Total number of acute iTTP episodes	224	
Age at time of diagnosis of first acute iTTP episode, y		
Median	33	
Range	12–64	
Frequency of all acute episodes per patient		
Median, total	2	
Range	1–21	
Median, female	2.0	
Median, male	2.5	
Observation time, y		
Median	8.3	
Range	0.4–31.9	

Abbreviation: iTTP, autoimmune thrombotic thrombocytopenic purpura.

Severity of Acute Bouts and Therapy

Detailed data for therapy was obtainable in 219 (►**Fig. 2**) and for severity in 213 (►**Supplementary Table S1**, available in the online version) of 224 acute episodes in 70 iTTP patients. No severity and therapy data were accessible in 3 iTTP patients in 4 acute bouts. Thus, detailed data for clinical severity as well as therapy in the respective acute bout were available for 211 of 224 acute episodes in 70 iTTP patients.

Most common were mild symptoms or signs such as fatigue and drowsiness (43.5%), headaches (26.8%), petechiae (27.3%), haematoma (23.4%), vertigo (12.9%), nausea (7.2%) and abdominal pain (6.7%). Among the more severe symptoms, impairment of consciousness (15.8%), dyspnoea (12.9%), acute renal failure (9.6%) and haematuria (11.5%) were most frequent. When counting neurological abnormalities together, they appeared in 34.4% of all bouts.

During the first iTTP manifestation, the proportion of severe episodes (50%) was higher than during 1st relapse (19%) and all subsequent relapses (13%) (►**Fig. 3**). Similarly, moderately severe bouts were more common during 1st acute episodes (32%) as compared with 1st (22%) and later (10%) relapses. In contrast, mild bouts and mere laboratory abnormalities were more common during 1st relapse (56%) and further relapses (76%) than during initial manifestation (17%) (►**Fig. 3** and ►**Supplementary Table S1**, available in the online version).

Plasma products were used in 191 of the 219 acute episodes (87%), prednisolone was administered in 188 (86%) and rituximab in 69 (32%) acute bouts. Overall, patients received 12 PEX procedures (median; range, 1–100, IQR, 6–23) for an acute bout. In the first acute episode, 62 (91.2%) iTTP patients received 21.5 PEX procedures (median; range, 2–100, IQR, 12–30). An acute iTTP bout lasted for 29 days (median; range, 1–160 days, IQR, 14–30 days).

Sixty-nine of of 219 acute bouts in 37 iTTP patients were additionally or exclusively (8 acute bouts in 3 different iTTP patients) treated with rituximab, whereas 150 acute bouts in 33

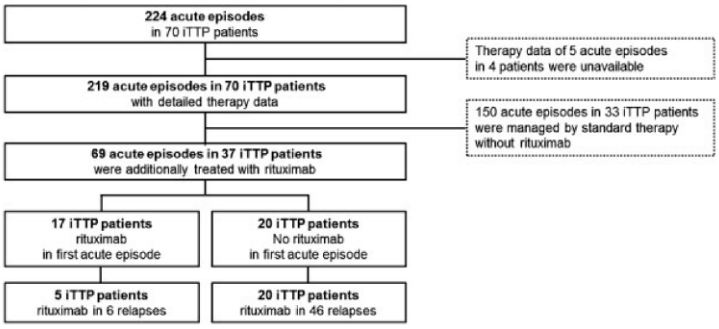

Fig. 2 Treatment of acute autoimmune thrombotic thrombocytopenic purpura (iTTP) episodes.

iTTP patients were not (►**Fig. 2**). Therefore, 31.5% of all acute TTP bouts were treated with rituximab in 53% of iTTP patients.

In this cohort, rituximab has exclusively been used in acute iTTP bouts.

The main indication for rituximab therapy was a refractory episode (58 acute episodes in 37 patients); a second indication was a high tendency to relapse (8 acute bouts in 4 patients).

Rituximab therapy was applied in acute iTTP events of all different clinical severity scores. Forty-three per cent of all bouts with severity score of 0, 26% of all mild bouts, 22% of all moderate bouts, 43% of all severe bouts and one of the two lethal bouts were treated with additional rituximab (►**Supplementary Table S2**, available in the online version).

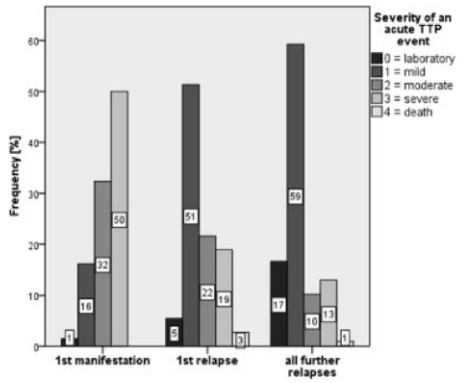

Fig. 3 Frequency of the different clinical severity scores at first, second and all following bouts in 70 autoimmune thrombotic thrombocytopenic purpura (iTTP) patients. During the first iTTP manifestation, the proportion of severe episodes was higher than during 1st relapse and all subsequent relapses. Absolute numbers in a total of 219 bouts: 1st manifestation: laboratory (0): $n = 1$, mild (1): $n = 11$, moderate (2): $n = 22$, severe (3): $n = 34$; 1st relapse: laboratory (0): $n = 2$, mild (1): $n = 19$, moderate (2): $n = 8$, severe (3): $n = 7$, death (4): $n = 1$; all further relapses: laboratory (0): $n = 18$, mild (1): $n = 64$, moderate (2): $n = 11$, severe (3): $n = 14$, death (4): $n = 1$.

Relapse Rate and Influencing Factors

Fifty-nine per cent of all 70 iTTP patients had at least one relapse independent of their therapy in acute episodes. Relapse rate (►**Fig. 4**) is defined as acute recurrent disease episodes in survivors of an initial iTTP bout per 100 patient-months of follow-up. The relapse rate of the whole group was 2.6% per month. Fifty-four women had a relapse rate of 2.4% per month and 16 men of 3.5% per month. Thus, men have a 1.5 (95% CI, 1.11–2.01; $p = 0.009$) times higher risk to relapse than women.

We analysed 219 acute episodes concerning possible risk factors for relapses. Smoking status seemed to be associated with higher relapse rate, whereas co-morbidities, including other autoimmune diseases and obesity, did not have an influence on the relapse rate when corrected for gender. With increasing age relapse rate declined.

Relapse rate after acute episodes treated with rituximab was 2.3% per month in comparison to 2.6% per month in acute bouts not treated with rituximab (►**Fig. 4**, Anderson–Gill model, $p = 0.729$, relative risk, 0.945, 95% CI, 0.687–1.30). Accordingly, rituximab had no significant influence on the relapse rate.

Relapse-Free Survival in 45 Patients with First iTTP Manifestation Since 2003

For this analysis, we considered all 45 patients with an initial acute iTTP bout since 2003, the time point when rituximab was first used for this indication at our institution. Seventeen iTTP patients received rituximab during their first acute episode and 20 iTTP patients only during relapses (►**Fig. 2**). We investigated the relapse-free survival time of these 45 iTTP patients, 17 receiving rituximab compared with 28 not receiving rituximab during their first acute iTTP bout (►**Fig. 5**). Assessing the relapse-free survival after a first acute episode using Kaplan–Meier analysis, no significant difference between both groups of patients, those treated versus those not treated with rituximab (log-rank test, p-value = 0.131) was evident (►**Fig. 5**). Relapse rate in iTTP treated with rituximab in the initial acute episode was 29% (5 out of 17) and 50% (14 out of 28) in those who did not. The event-free survival in patients treated or not treated with rituximab after 1 year was 94 and 82%, respectively, and 79 versus 57%, respectively, after 1,000 days. The median event-free time

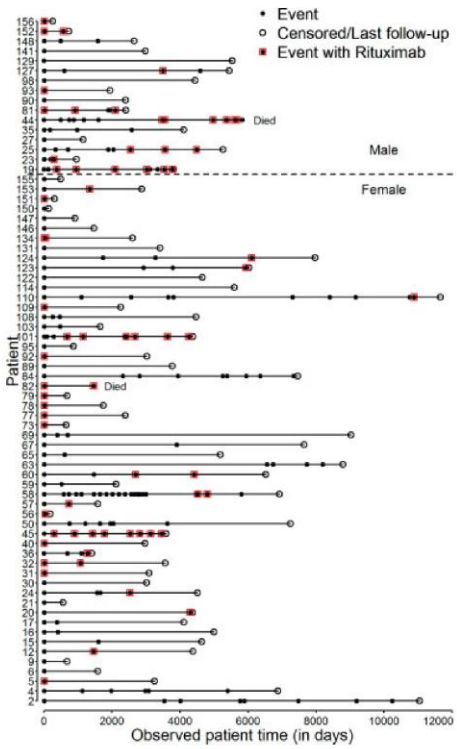

Fig. 4 Event history of all autoimmune thrombotic thrombocytopenic purpura (iTTP) patients with detailed therapy data. Acute iTTP episodes of all 70 patients from the first day of first acute thrombotic thrombocytopenic purpura (TTP) episode until end of observation time in days. Patients were pseudonymized with a number code. Men are listed (above dotted horizontal line no. 19–156) in the upper part and women in the lower part of the figure (below dotted line no. 2–155). Acute iTTP episodes are represented by a black dot. If being treated by rituximab, this circle is bordered by red rectangles. Empty circles represent the last day of observation. Two patients died during a relapse (Died): including one woman following her first acute TTP relapse (no. 82). She had denied plasma products for religious beliefs. One man did not survive his 13th acute TTP episode (no. 44).

until first relapse was 1,337 days in patients not treated with rituximab. This value cannot be determined for patients treated with rituximab because less than half of them had a relapse.

Discussion

Since 2003, rituximab was used in 69 acute episodes as second-line medication in 37 of our 70 patients, generally because they were refractory to standard treatment or showed early relapses. Rituximab has been increasingly used in iTTP over the past 15 years,[7,13,14] albeit the indications (refractory disease, upfront treatment in all patients, pre-emptive treatment in survivors with recurring severe ADAMTS13 deficiency) are heterogeneous and still debated.[15] Rituximab was initially used for patients with refractory acute TTP and those with early relapses, and was generally considered to be effective.[14,15] In our retrospective cohort, 59% of all 70 iTTP patients had a relapse, independent of whether rituximab was added or not. This is higher than the 40% reported by Coppo and Froissart and Ferrari et al.[16,17] Relapse rate was 29% in 17 iTTP patients treated with rituximab during their first acute episode, and 50% in those who did not. Chemnitz et al observed a relapse rate of 25% in their 12 rituximab-treated patients over a 50-month period.[18] Scully et al found a relapse rate of only 10% in her upfront-rituximab-treated cohort, which compared favourably with a rate of 57% in a historical control group not having received rituximab.[19] Similar data, relapse rates of 43% without versus 12.5% with rituximab, were reported by Page et al in a smaller cohort but with simultaneously treated control group.[20] According to accumulated data from various cohorts, rituximab may decrease the frequency of subsequent relapses; nonetheless, we can detect only a weak tendency and no significant effect in our cohort. The influence of rituximab seems to be greater in the first year after the initial episode. Our data are similar to that reported by Froissart et al finding no significant difference in the relapse rate of their refractory patients treated versus not treated with rituximab.[21] Advantage of rituximab seems to lie in a faster recovery and possibly less recurrences during the first year.[21–25] We can confirm that, in our iTTP patients the event-free-survival after 1 year was 94% (treated with rituximab) versus 82% (not treated with rituximab).

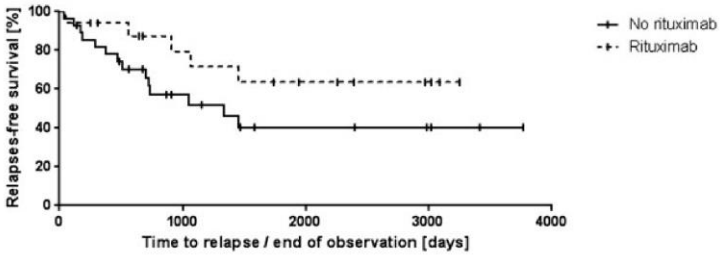

Fig. 5 Kaplan–Meier estimates of relapse-free survival of 45 autoimmune thrombotic thrombocytopenic purpura (iTTP) patients with a first disease bout since 2003. Relapse-free survival of iTTP patients receiving rituximab ($n = 17$) (upper dotted curve) compared with iTTP patients who did not receive rituximab ($n = 28$) (lower curve) during their first acute thrombotic thrombocytopenic purpura (TTP) bout. Vertical bars denote censored patients not having suffered from relapse. There was no statistical difference between these groups (p-value $= 0.131$).

Severe ADAMTS13 deficiency is pathophysiologically strongly linked to the development of clinical disease manifestations. Furthermore, high anti-ADAMTS13 immunoglobulin G (IgG) antibody titers are associated with increased mortality.[26] In turn, decrease of anti-ADAMTS13 IgG is directly associated with rituximab therapy,[25] suppression of B-lymphocytes being detectable for 9 to 15 months. Hence, relapse rate must be carefully watched after this time period.[23]

As it is necessary to achieve remission and reduce relapse frequency, it is desirable to prevent life-threatening relapses at all. In our patients, the initial iTTP bouts were more severe than the later occurring relapses, which have been reported by earlier investigators as well.[27] This may not be due to the disease itself but rather to increased awareness following the initial diagnosis. Our patients were regularly seen and amenable to early treatment. In a few patients with frequent relapses, rituximab was given after detection of defined laboratory abnormalities even before clinical symptoms arose which led to reduced need of PEX. Early administration of rituximab leads to faster remission and lower number of necessary PEX sessions.[22] This finally raises the question whether rituximab should be given to any patient with persisting or reappearing severe ADAMTS13 deficiency even before any fall in platelet count or LDH increase is apparent. Hie et al showed that pre-emptive rituximab treatment in asymptomatic survivors of at least one iTTP bout seemed to reduce relapses as compared with patients not receiving rituximab[28] however, this pre-emptive treatment has been called into question by others.[15]

Moreover, gender plays an important role regarding relapse rate and severity of acute bouts. Generally, men are affected by severe autoimmune diseases more rarely than women.[29,30] According to our results, men suffered significantly more and also more serious relapses than women. This is similar to other autoimmune diseases such as multiple sclerosis and in systemic lupus erythematous with men suffering from more severe disease course as compared with women.[30,31] A significantly higher tendency to relapse for men has also been described by Fakhouri et al.[23] Further investigation needs to evaluate whether gender-adapted treatment is necessary.

Strengths and Limitations of this Study

Our study has several limitations. One is the retrospective data acquisition. In hindsight, missing data or further desirable information could not be collected. On the other side, the long observation time was an advantage over cohorts with shorter follow-up periods. This enabled us to generate relevant results regarding relapse rates. Another limitation is a treatment bias regarding the use of rituximab. Rituximab was predominantly used in iTTP bouts, resistant to conventional PEX and corticosteroid treatment or in patients with a high tendency to relapse. Strength of our study is that iTTP patients given rituximab were compared with simultaneously recruited control iTTP patients, although the two cohorts are small for statistical analysis and not matched by age or sex. A randomized, prospective controlled trial would still be desirable to clarify the role of rituximab but it seems rather unlikely that such a trial will be performed shortly.

In conclusion, we did not detect a significant advantage of rituximab regarding relapse rate, neither during the time between initial acute episode and the first relapse, nor in long-term observation including all acute episodes. Nevertheless, we confirm that rituximab can help to achieve remission in refractory iTTP. Interestingly, we found that men suffer significantly more frequent and considerably more serious acute relapses. Furthermore, initial episodes are characterized by more severe clinical signs compared with the less severe relapses.

What is known about this topic?

- Rituximab has been increasingly used in iTTP over the past 15 years.
- The first and commonly accepted indication concerns patients refractory to standard treatment with PEX and corticosteroids. Recently, upfront rituximab in any patient with acute iTTP has been proposed based on data suggesting faster remission and shorter hospital stay. Lastly, prophylactic rituximab has been given to survivors of an acute iTTP with recurrent or persistent severe ADAMTS13 deficiency to avoid relapses. No formal indication has been approved.

What does this paper add?

- First large German cohort of 70 iTTP patients examined for factors influencing the relapse rate.
- Rituximab has no significant effect on the long-term relapse rate in our cohort.
- We report that men suffer significantly more frequent and considerably more serious acute episodes than women.
- Initial acute episodes are characterized by more severe clinical signs compared with the less severe relapses.

Authors' Contributions

T. Falter: Study concept and design, data analysis, writing of the manuscript and approval. S. Herold: Data acquisition and analysis, writing of the manuscript and approval. V. Weyer: Statistical advice, revision of the manuscript and approval. C. Scheiner: Statistical advice, revision of the manuscript and approval. V. Schmitt: Data acquisition, revision of the manuscript and approval. X. Messmer: Data acquisition and approval. C. von Auer: Revision of the manuscript and approval. P. Wild: Revision of the manuscript and approval. K. Lackner: Revision of the manuscript and approval. B. Lämmle: Data compilation, writing of the manuscript, revision of the manuscript and approval. I. Scharrer: Study initiation, revision of the manuscript, writing of the manuscript and approval.

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Conflict of Interest
The authors declare that they have no conflicts of interest relevant to the manuscript. I. Scharrer is a member of the Data Safety Monitoring Board in the BAX 930 study (investigating recombinant ADAMTS13 infusion in hereditary TTP). She received travel and accommodation support for participating at scientific congresses or meetings from Bayer and NovoNordisk. B. Lämmle is chairman of the Data Safety Monitoring Board in the BAX 930 study (investigating recombinant ADAMTS13 infusion in hereditary TTP). He is on the Advisory Board of Ablynx for the development of caplacizumab. He holds a patent on ADAMTS13 and received travel and accommodation support for participating at scientific congresses or meetings from Baxalta, Siemens, Alexion, Ablynx and Bayer and speaker's fees from Siemens, Bayer and Alexion.

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7.4. Originalarbeit IV.

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Influence of Personality, Resilience and Life Conditions on Depression and Anxiety in 104 Patients Having Survived Acute Autoimmune Thrombotic Thrombocytopenic Purpura

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Abstract: Autoimmune thrombotic thrombocytopenic purpura (iTTP) is a life-threatening, relapsing disease in which an acquired deficiency of the enzyme ADAMTS13 leads to generalised microvascular thrombosis. Survivors have a high prevalence of depression and impaired cognitive function. The aim of this study was to determine whether life circumstances and personality have an influence on the development and severity of depression and anxiety in iTTP patients and how they impact the quality of life. With validated questionnaires, we examined the prevalence of depression and anxiety symptoms in 104 iTTP patients, as well as parameters of subjective cognitive deficits, quality of life, attitude to life and resilience. iTTP patients had significantly more depressive symptoms ($p < 0.001$), a tendency to have anxiety disorders ($p = 0.035$) and a significantly worse cognitive performance ($p = 0.008$) compared to the controls. Sex, age, physical activity and partnership status had no significant influence on depression, whereas the number of comorbidities did. Lower scores of resilience, attitude to life and quality of life were reported by patients compared to controls. iTTP patients had a high prevalence of depression and anxiety, as well as a more negative attitude to life and low resilience. Resilience correlated negatively with the severity of the depression. Furthermore, quality of life and cognitive performance were significantly reduced.

Keywords: thrombotic thrombocytopenic purpura; depression; resilience; quality of life

1. Introduction

Autoimmune thrombotic thrombocytopenic purpura (iTTP) is a potentially life-threatening, relapsing disease in which an acquired deficiency of the von Willebrand factor (VWF)-cleaving protease, ADAMTS13, leads to generalised microvascular thrombosis in various organs [1]. The characteristic features are thrombocytopenia due to the consumption of platelets and microangiopathic haemolytic anaemia with destruction of erythrocytes [2]. As soon as the laboratory parameters return to normal after treatment of an acute bout, the patient is often regarded as cured but lives with the risk of suffering an acute relapse at any time [3]. In recent years, some studies have shown that iTTP is much more than just an acute disease; not

only potential relapses but also long-term consequences of the past acute episode should be in focus. Besides neurological impairments [4–7], depression is a prevalent sequela [6–10]. The occurrence of depression and anxiety disorders has been documented in numerous other acute and chronic diseases, e.g., stroke, multiple sclerosis and cancer [11–15]. Depression itself is considered a risk factor for cardiovascular disease [16] and leads to increased morbidity and mortality, regardless of its severity [15,17]. Furthermore, depression causes a reduced quality of life for patients and lower resilience. In turn, individuals with low resilience are more prone to develop psychiatric disorders [18]. However, resilience is also significantly influenced by other factors, such as alexithymia [19].

We [10], as well as others [6,9,20], have shown that the prevalence of depression is significantly increased in patients that have survived acute iTTP. In addition, our results revealed that the severity of the acute iTTP episode is not the determining factor for the development and severity of depression [10].

The aim of the present study was to determine whether life circumstances (e.g., partnership, employment and physical activity), personality and resilience are associated with the development and severity of depression and anxiety in iTTP patients and how they influence their quality of life.

2. Materials and Methods

The results are part of a five-year prospective cohort study that was approved by German law (Landeskrankenhausgesetz §36 and §37) in accordance with the Declaration of Helsinki and by the local Ethics Committee of "Landesärztekammer Rheinland-Pfalz" (837.265.14 (9504-F)), where all participants gave written consent to participate.

The study was divided into two main themes. The first part referred to evaluations in 2013 and 2014 that have already been published [10]. In brief, the iTTP patients displayed a high prevalence of depression and cognitive deficits via self-reporting questionnaires. However, we did not detect a significant correlation between the severity of depression or cognitive deficits and the number or severity of acute TTP episodes. Nevertheless, we could demonstrate a highly significant correlation between the severity of depression and the degree to which cognitive performance was reduced [10].

The second part had a focus on the long-term psychological consequences, where the personality structure and the influence on the quality of life were examined in more detail here (Figure S1).

In 2015 and 2016, using validated questionnaires, we examined the prevalence of depressive (PHQ-9) and anxiety symptoms (GAD-7) in 104 iTTP patients, as well as parameters of subjective cognitive deficits (FLei), resilience (RS-11), attitude to life (LOT-R) and quality of life (QLQ-C30) at two observation points one year apart. At the second observation time, an age- and sex-matched healthy control group was simultaneously interviewed.

2.1. Patients and Controls

The patient cohort for this study was recruited from the iTTP patients that were treated directly at the University Hospital Mainz, as well as from external patients for whom the University Hospital Mainz was asked for medical advice by external clinics. The external patients that presented themselves personally at the University Hospital Mainz at least once were asked to participate in the study. Since October 2012, all patients over 18 years of age with a confirmed iTTP diagnosis (defined as microangiopathic haemolytic anaemia, thrombocytopenia (<150,000/µL), severe acquired ADAMTS13 deficiency (activity < 10%) and an ADAMTS13 inhibitor (>0.5 Bethesda units/mL)) in the acute TTP episode have been included.

The healthy controls were 300 randomly selected people that were age- and gender-matched to the iTTP collective, whose contact details were received from the residents' registration office. We received 134 evaluable questionnaires.

2.2. Psychometric Assessment

TTP patients were invited to participate in the study twice with an interval of 10 to 12 months. At both time points, psychometric questionnaires were either sent by regular mail to the patients' home or directly given to patients when they presented at the outpatients ward.

One patient was excluded from this study (in 2015/2016) because of an inability to answer the questionnaires after having suffered from ischemic brain damage during an acute TTP episode.

2.2.1. Patient Health Questionnaire 9 Items (PHQ-9)

The Patient Health Questionnaire 9 (PHQ-9) was developed in 2001 by Spitzer et al. and is indicated for the self-assessment of depressive symptoms and their classification into degrees of severity [21]. It consists of nine questions, each of which is attributed 0, 1, 2 or 3 points. The final score is calculated from the sum of all answers. A high score indicates that patients often show depressive symptoms. If the patient receives 0 to 4 points, it can be assumed that there is no depression. Mild depressive symptoms are present at 5 to 9 points, moderate symptoms at 10 to 14 points and moderate-to-severe depression at 15 to 19 points. A score $\geq$ 20 points signals severe depressive symptomatology. The presence of major depression can be assumed at a cut-off of $\geq$10 points.

2.2.2. Generalized Anxiety Disorder 7 (GAD-7)

The "Generalized Anxiety Disorder 7", which is a self-assessment questionnaire with seven items, was developed to diagnose and classify generalised anxiety disorders [22]. The GAD-7 examines the symptoms of anxiety, such as nervousness or irritability in seven items. The patient must evaluate how often these symptoms have been experienced in the last 2 weeks. Depending on the answer, the patient receives between 0 and 3 points. The sum of all seven items corresponds to the total score. If the total score is between 0 and 4 points, no anxiety disorder can be assumed. A score of 5 points or more indicates a mild anxiety disorder, 10 points or more indicates a moderate anxiety disorder and 15 points or more indicates a severe anxiety disorder.

2.2.3. FLei

Cognitive deficits were assessed using the German questionnaire for complaints of cognitive disturbances (FLei), which is a self-report measure with 30 items covering the domains of deficient attention, memory and executive functions, with 10 items each. All items are rated on a five-point Likert-scale (0 = at no time; 4 = very frequent). Accordingly, the total score for all 30 items ranges between 0 and 120 points. The internal consistencies of the three subscores (Cronbach's alpha and split-half reliability) are all >0.87 [23]. Healthy controls reported in the literature showed a mean of 29.1 (SD 18.7), whereas controls with major depression (ICD.10) had a mean of 56.5 (SD 23.1) [23].

2.2.4. Resilience Scale 11 (RS-11)

The Resilience Scale 11 (RS-11) was developed as a tool to measure the mental resistance of patients [24]. The self-assessment questionnaire consists of 11 questions, each of which is rated with 1 to 7 points. From these scales, an overall score is formed, with values from 11 to 77. The higher the total score, the higher the presumed resilience of the respondent.

2.2.5. Life Orientation Test–Revised (LOT-R)

The Life Orientation Test–Revised (LOT-R) is a questionnaire with 10 items, each with five possible answers, which serves to assess the attitude to life. It evaluates general character features, such as the tendency toward optimism and pessimism, for both of which, a subscore is given. In addition, an overall score can be calculated [25].

2.2.6. Quality of Life Questionnaire C 30 (QLQ-C30)

The Quality of Life Questionnaire C 30 (QLQ-C30) was developed in 1993 by the European Organisation for Research and Treatment of Cancer to specifically evaluate the quality of life of cancer patients [26,27]. Fifteen subscales are formed from the 30 items. The subscales consist of five function scales (physical, role, cognitive, emotional and social function), three symptom scales (fatigue, pain and nausea or vomiting) and a global health status/quality of life scale, as well as six individual items with specific symptoms (dyspnea, loss of appetite, insomnia, constipation and diarrhea, and a question on the financial impact of the disease). Each item has four response alternatives, except for the global health status/quality of life scale, which has response options ranging from 1 to 7.

2.3. Covariates

In addition to the questionnaires, personal information, such as age, gender and life circumstances such as partnership and number of children, were also collected. Furthermore, data on physical fitness and other chronic and acute illnesses were obtained. The participants were able to specify their physical fitness themselves with the help of five predefined answer options (from not at all or only a little bit (1–2 times per month) to extremely active (more than 5 times per week)). Fifteen comorbidities were specifically asked for and further comorbidities could be indicated.

2.4. Statistical Analyses

Statistical analyses were performed using SPSS version 22.0 (IBM GmbH, Ehningen, Germany). Missing data were imputed using median imputation. The descriptive statistics included frequency, mean, standard deviation, median, interquartile range (IQR), minimum and maximum. The differences between the two groups were tested using Student´s *t*-test for normally distributed data and the non-parametric Mann–Whitney *U* test for non-normally distributed data. For comparing changes in the different scores of patients who completed both surveys (in 2015 and 2016), a dependent *t*-test was used, as well as the dependent Wilcoxon test. Spearman's rank correlation coefficient (r_s) was calculated to estimate the relationship between depressive symptoms and resilience, respectively. The correlations between age, gender, comorbidities, physical activity and depressive symptoms were determined using Pearson's correlation coefficient (r). Any p-values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Study Population

From June 2015 until July 2016, 147 patients with an acquired TTP that was diagnosed prior to starting this study were asked to participate. Between the 2015 and 2016 surveys, five of the 147 participants were lost to follow-up. Accordingly, 142 TTP patients were sent the questionnaires in the 2016 survey, about one year after the first inquiry. We received 89 responses in the 2015 survey, with 89 of those being evaluable for RS-11, 88 for PHQ-9, 87 for GAD-7 and LOT-R and 85 for FLei (Figure 1). Eighty-four responses were obtained in the 2016 survey, with 83 of those being evaluable for Flei, 81 for PHQ-9, RS-11 and QLQ-C30 and 80 for GAD-7 and LOT-R (Figure 1).

Overall, we received responses from 104 individual iTTP patients, 69 answered both surveys, 20 participated only in 2015 and 15 only in 2016 (Figure 1). The depression, anxiety, impairment of cognitive performance, resilience, attitude of life and quality of life results of the iTTP patients were compared with those from 134 healthy controls.

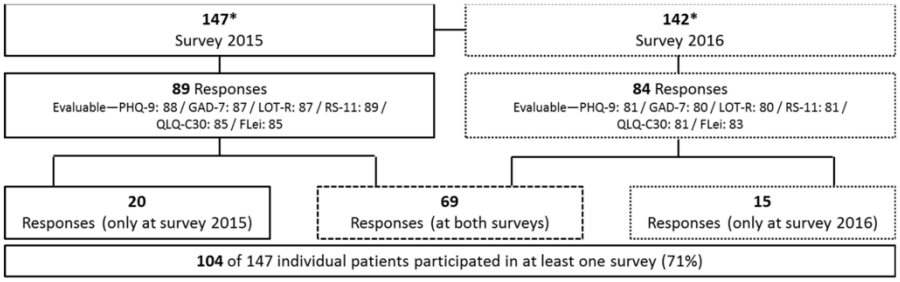

Figure 1. Patient recruitment and response rates in two surveys of the cohort of autoimmune thrombotic thrombocytopenic purpura (iTTP) patients from Mainz. A total of 147 eligible iTTP patients in remission were invited to fill in the various questionnaires used in two surveys each (2015 and 2016). * Between the first and second survey five patients were lost to follow-up. Questionnaires concerned: depression (PHQ-9), anxiety (GAD-7), attitude of life (LOT-R), resilience (RS-11), quality of life (QLQ-C30) and cognitive disturbance (FLei).

3.2. Patient Characteristics

A total of 147 (2015) and 142 (2016) iTTP patients could be reached for the surveys. The response rate was 60% in the 2015 survey and 59% in the 2016 survey (Figure 1). The characteristics of the patients and the healthy controls are shown in Table 1.

Table 1. Characteristics of the responding and evaluable autoimmune thrombotic thrombocytopenic purpura (iTTP) patients in both surveys and of the healthy controls.

Heading	iTTP Patients		Healthy Controls
Time of survey	2015	2016	2016
Number (*n*)	89	84	134
Gender and age			
Female	69 (76%)	69 (82%)	108 (81%)
Male	20 (24%)	15 (18%)	26 (19%)
Age (years) median (min, IQR, max)	48 (18, 37–59, 86)	51 (21, 38–59, 87)	48 (19, 30–60, 79)
Data for age missing	6	7	0
Current partnership			
Yes	62 (73%)	60 (75%)	96 (74%)
No	23 (27%)	20 (25%)	34 (26%)
Data missing	4	4	4
Occupation, BMI, smoking status			
Employed	45 (51%)	41 (50%)	72 (55%)
Studying	3 (3%)	1 (1%)	14 (11%)
Retired	26 (30%)	22 (27%)	27 (21%)
Unemployed	2 (2%)	4 (5%)	2 (1%)
Working at home	4 (5%)	7 (8.5%)	5 (4%)
Other	8 (9%)	7 (8.5%)	10 (8%)
Data missing	1	2	4
BMI median (min, IQR, max)	26 (18, 23–31, 48)	28 (18, 24–32, 47)	24 (18, 21–26, 42)
Obesity (BMI $\geq$ 30)	24 (27%)	29 (35%)	12 (9%)
Data missing	0	2	4
Smoking	22 (25%)	21 (25%)	18 (14%)
Data missing	0	1	2
Physical activity			
Hardly active (1–2×/month)	25 (29%)	32 (39%)	22 (17%)
Quite active (3–4×/month)	10 (12%)	13 (16%)	24 (18%)
Active (1–2×/week)	33 (39%)	24 (30%)	45 (34%)

Table 1. *Cont.*

Heading	iTTP Patients		Healthy Controls
Very active (3–4×/week)	14 (16%)	8 (10%)	32 (24%)
Extremely active (>5×/week)	3 (4%)	4 (5%)	9 (7%)
Data missing	4	3	2
Number of comorbidities [1]			
0	20 (22%)	10 (12%)	48 (36%)
1	25 (28%)	20 (24%)	43 (33%)
2	14 (16%)	23 (28%)	18 (14%)
≥3	30 (34%)	30 (36%)	23 (17%)
Data missing	0	1	2

[1] Includes cardiovascular diseases, hypertension, gastrointestinal diseases, rheumatoid arthritis, diabetes mellitus, skin diseases, metabolic disorders, allergies, multiple sclerosis, chronic pulmonary diseases, chronic pain, thyroid diseases, obesity, cancer and other.

About 80% of the patients were female and the median ages were 48 and 51 years in 2015 and 2016, respectively. Half of the participating patients were employed and one third were retired. The majority of patients lived in a partnership and 63% and 65% in 2015 and 2016, respectively, had children. One-third of patients each took part in low, intermediate or high physical activity. The median body mass index (BMI) was 26 and 28 kg/m^2, respectively, in the two surveys, with 27% and 35% being obese. Overall, the iTTP collective had several other diseases besides iTTP (78% had comorbidities in 2015 and 88% in 2016). On average, the iTTP patients had one additional disease in 2015 (min 0, IQR 1–3, max 9) and two additional comorbidities in 2016 (min 0, IQR 1–3, max 9). The control group had substantially fewer diseases (median 1, min 0, IQR 0–2, max 7). The comorbidities that were explicitly asked for were chronic heart diseases, hypertension, gastrointestinal diseases, rheumatoid arthritis, diabetes mellitus, skin diseases, metabolic disorders, allergies, multiple sclerosis, chronic pulmonary diseases, chronic pain, thyroid diseases, obesity and cancer. In addition, comorbidities not listed could be indicated. Both in iTTP patients and the control group, the most frequent health problems were hypertension and thyroid diseases, followed by allergies. Compared to the control collective, the iTTP patients were significantly more overweight, were more often smokers and had more comorbidities (Table 1).

3.3. Depression (PHQ-9)

In 2015, 54 (61.4%) of 88 iTTP patients were scored as having current depressive symptoms by the PHQ-9 (score $\geq$ 5) and the proportion of patients with major depression (score $\geq$ 10) was 21.6%. The median score was 5 (IQR 2–10), ranging from 0 to 23 (Figure 2a). Thirty-four (38.6%) patients had no depression, 31 (35.2%) had mild depression, 13 (14.8%) had moderate depression, nine (10.2%) had moderate-to-severe depression and 1 (1.1%) had severe depression.

In 2016, 51 (63.0%) of 81 iTTP patients were scored as having current depressive symptoms by the PHQ-9 (score $\geq$ 5) and the proportion of patients with major depression (score $\geq$ 10) was 34.5%. The median total score was 7 (IQR 2.5–12.5), ranging from 0 to 23 points (Figure 2a). Regarding the severity of depression, 30 (37.0%) patients had no depression, 23 (28.4%) had mild depression, 14 (17.3%) had moderate depression, 13 (16.0%) had moderate-to-severe depression and 1 (1.2%) had severe depression.

Forty-five of 133 (33.8%) healthy controls had depressive symptoms as scored by the PHQ-9 (score $\geq$ 5) (Figure 2a). Six (4.6%) of the 133 controls had clinically relevant depression (score $\geq$ 10). The median total score was 3 (IQR 1–6), ranging from 0 to 18 points (Figure 2a). The prevalence of depression in iTTP patients was significantly higher in both surveys (2015 $p < 0.001$; 2016 $p < 0.0001$) than in the controls (Figure 2a). No difference in the prevalence or severity of depression in the iTTP patients was found between the two surveys.

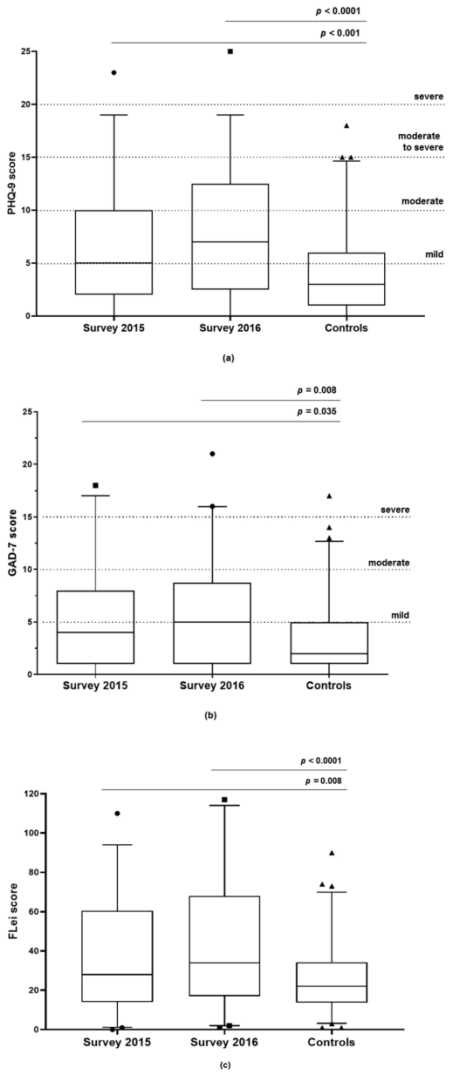

Figure 2. Results of the depression (PHQ-9), anxiety disorder (GAD-7) and cognitive performance (FLei score) questionnaires from the iTTP patients in two surveys (2015 and 2016) and the healthy controls (median, box 25th and 75th percentiles, whiskers 2.5th and 97.5th percentiles, ●, ■, ▲ denote outliers above 97.5th percentiles or below 2.5th percentiles outliers). (**a**) PHQ-9: For the first survey (n = 88), the median evaluated score was 5 (IQR 2–10), for the second survey (n = 81), the median score was 7 (IQR 2.5–12.5), and for the healthy controls, the median score was 3 (IQR 1–6). (**b**) GAD-7: For the first survey (n = 87), the median evaluated score was 4 (IQR 1–8), for the second survey (n = 80), the median score was 5 (IQR 1–8.75), and for the healthy controls (n = 131), the median score was 2 (IQR 1–5). (**c**) FLei: For the first survey (n = 85), the median evaluated score was 28.0 (IQR 14–60.5), for the second survey (n = 81), the median score was 34.0 (IQR 17–68), and for the healthy controls (n = 130), the median score was 22.0 (IQR 13.75–34.25).

3.4. Anxiety Disorder (GAD-7)

In 2015, 49 (56.3%) of 87 iTTP patients had no symptoms of anxiety, whereas 21 (24.1%) had mild anxiety (score 5–9), 12 (13.8%) had moderate anxiety (score 10–14) and five (5.7%) had severe anxiety (score 15–21). The median evaluated score was 4 (IQR 1–8), ranging from 0 to 18 (Figure 2b).

In 2016, 37 (46.3%) of 80 iTTP patients had no symptoms of anxiety, whereas 29 (36.3%) patients had mild anxiety (score 5–9), 11 (13.8%) had moderate anxiety (score 10–14) and three (3.8%) had severe anxiety (score 15–21). The median evaluated score was 5 (IQR 1–9), ranging from 0 to 21 (Figure 2b). Ninety-five of the 132 controls (72.0%) did not show any symptoms of anxiety (Figure 2b). The prevalence of anxiety disorders in the overall iTTP cohort was higher in both surveys (2015 $p < 0.035$; 2016 $p < 0.008$) than in the control group (Figure 2b). In particular, the proportion of clinically relevant anxiety disorders (score ≥ 10) in the iTTP cohort was significantly higher in 2015 (19.5%) and in 2016 (17.6%) than in the control group (8.4%).

3.5. Cognitive Performance (FLei Score)

Eighty-five iTTP patients in 2015 and 81 in 2016 were evaluable for their cognitive performance using FLei (Figure 2c). The total scores in both surveys were normally distributed and showed a median of 28.0 (IQR 14–60.5) in the 2015 survey and a median of 34.0 (IQR 17–68) in the 2016 survey, ranging from 0 to 117 (Figure 2c). Cognitive performance was significantly worse for iTTP patients in both surveys ($p = 0.008$ for 2015, $p < 0.0001$ for 2016) in comparison to the healthy cohort (median 22.0, IQR 13.75–34.25) (Figure 2c).

3.6. Resilience (RS-11)

The 89 iTTP patients in the first survey in 2015 showed a median score of 60 (min 22, IQR 49.5–68.5, max 77) and the 81 iTTP patients in the second survey 2016 showed a median score of 55 (min 21, IQR 45–66, max 77) (Figure 3a). The control collective of 129 persons had a median score of 64 (min 33, IQR 56–69, max 77) (Figure 3a). Thus, the survivors of iTTP, both in the first ($p < 0.04$) and second ($p < 0.0001$) surveys, exhibited a lower resilience than the control collective (Figure 3a).

3.7. Attitude to Life (LOT-R)

The questionnaire on the attitude to life (LOT-R) was answered by 87 patients in 2015 and 80 patients in 2016. The results in the categories of optimism, pessimism and the total score could be compared with 134 control persons. In the first survey, no significant difference ($p = 0.088$) between the patients (median 15, IQR 12–19) and controls (median 17, IQR 14–19) was found in the total score, but in the second survey, a significant difference ($p = 0.009$) between the patients (median 17, IQR 11–18) and controls was found (Figure 3b). In the optimism score, the patients showed significantly worse results than the control group in both rounds (2015 survey $p = 0.011$, 2016 survey $p = 0.006$) (Figure 3b). Within the pessimism score, no large differences between the patients and controls could be detected (2015 survey $p = 0.49$, 2016 survey $p = 0.63$) (Figure 3b).

3.8. Quality of Life (QLQ-C30)

Eighty-five TTP patients in 2015 and 81 patients in 2016 were evaluable regarding their quality of life using the QLQ-C30 (Figure 3c). They could be compared with 134 healthy controls (Figure 3c). In all five functional scales (physical, cognitive, role and social function $p < 0.0001$ for both 2015 and 2016; emotional function $p = 0.001$ for 2015/$p = 0.007$ for 2016), as well as in the global quality of life scale ($p = 0.001$ for 2015/$p = 0.007$ for 2016), the iTTP patients showed significantly worse results in both rounds than the control group (Figure 3c, not all five functional scales are shown).

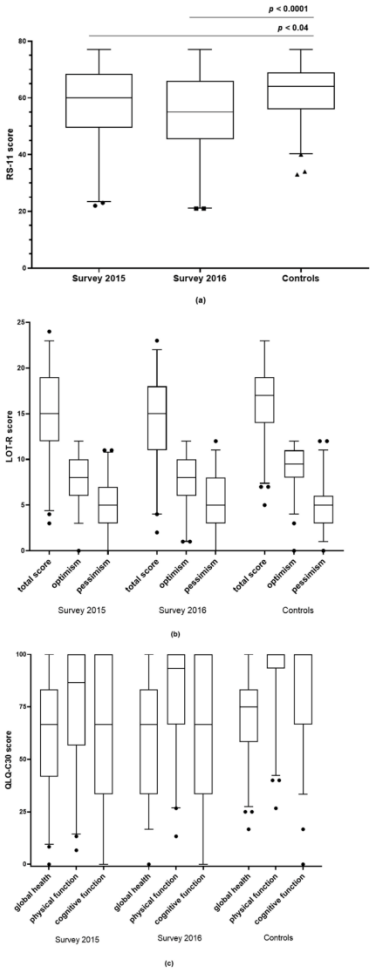

Figure 3. Results of the resilience (RS-11), attitude to life (LOT-R) and quality of life (QLQ-C30) questionnaires from the autoimmune thrombotic thrombocytopenic purpura (iTTP) patients in two surveys (2015 and 2016) and the healthy controls (median, box 25th and 75th percentiles, whiskers 2.5th and 97.5th percentiles, ●, ■, ▲ outliers above the 97.5th percentiles or below the 2.5th percentiles). (**a**) RS-11: For the survey in 2015, the median evaluated score was 60 (IQR 49.5–68.5), for the survey in 2016, the score was 55 (IQR 45–66), and for healthy controls, the score was 64 (IQR 56–69). (**b**) LOT-R: In the optimism score, the patients showed significantly worse results than the control group in both rounds (2015 survey $p = 0.011$, 2016 survey $p = 0.006$). Within the pessimism score, no large differences between the patients and controls could be detected (2015 survey $p = 0.49$, 2016 survey $p = 0.63$). In the first round, no significant difference ($p = 0.088$) between the patients and controls was found in the total score, but in the second round, a significant difference ($p = 0.009$) between the patients and controls was found. (**c**) QLQ-C30: In the "global health", "physical function" and "cognitive function" scores, the patients had significantly worse results than the control group in both rounds (2015 and 2016 surveys $p < 0.0001$).

3.9. Correlation of Life Circumstances and Personality with Depression

Sex, age, physical activity and partnership status were not significantly correlated with depression. Using Pearson's correlation (age, physical activity, partnership status) and Mann–Whitney *U* analysis (sex), no significant correlation was established for any of these parameters in 2015 or 2016 with the degree of depression (PHQ-9 score) (Table S1). The comorbidities were associated with the PHQ-9 score. Only the number of co-morbidities was considered, not the specific diseases. If a patient had more co-morbidities, the PHQ-9 score showed a higher value, i.e., a more severe depressive state ($p = 0.015$ for 2015/$p = 0.006$ for 2016) (Table S1). Furthermore, the correlation between the QLQ-C30 score (quality of life) and the PHQ-9 score was significant ($p < 0.0001$ for both 2015 and 2016) (Table S1).

3.10. Correlation of Resilience with Depression

Our data revealed that the degree of depression (PHQ-9) was negatively associated with resilience (RS-11). Spearman's rank correlation coefficient (r_s) for 88 iTTP patients in the 2015 survey was -0.5346 ($p < 0.0001$), and for the 78 participants in the 2016 survey, $r_s = -0.6447$ ($p < 0.0001$). In Figure 4, the RS-11 and PHQ-9 data for 102 individual iTTP patients (only the first survey was considered for patients who participated in both surveys) revealed an r_s of -0.5878 ($p < 0.0001$) (Figure 4). Seventy iTTP patients without major depression (PHQ-9 score points < 10) had a median of 62 for the RS-11 score, which was comparable to the controls (median RS-11 score: 64, $p = 0.65$). The 32 iTTP patients with major depression (PHQ-9 score $\geq$10) had a median of 41.5 for the RS-11 score, which was significantly lower than that of the controls and the iTTP patients without major depression ($p < 0.0001$).

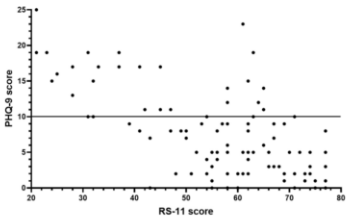

Figure 4. Correlation of the PHQ-9 score (depressive symptoms) with the resilience score. The correlation of the degree of depression (PHQ-9) with resilience (RS-11) was analysed for 102 iTTP patients ($r_s = -0.588$, $p < 0.0001$) (every iTTP patient was analysed only once, the first evaluation of those that participated in both surveys was considered). The horizontal line indicates the cut-off for major depression (PHQ-9 score $\geq$10).

4. Discussion

For a long time, the survival of acute iTTP bouts was the main concern, but in recent years, the long-term consequences in survivors of iTTP have become more important. The prevalence of major depression in our iTTP patients was 21.6% and 34.5% for 2015 and 2016, respectively, far above the prevalence in our population controls (4.6%) and the reported 12-month prevalence in the German population (9.3%) [28]. These results are consistent with our previous findings [10] and with other studies showing a significantly increased point prevalence of depression from 19% up to 65% in iTTP survivors [6,8,9,29,30]. A strong association between chronic physical illness and depression has been reported [15,31]. Independent from the disease, the rate of 21.1% mood disorders in patients is significantly higher than in healthy individuals with 9.4% [31]. In addition, anxiety disorders have been documented, for example, in patients after a heart attack or stroke and with cancer [15,31]. Anxiety disorders in those with serious illnesses are just as common (22.9%) as depression (21.1%) [31]. Within the general population, anxiety disorders are the most common

mental disorder, affecting about 15% [28]. Our examination of 87 iTTP patients revealed clinically relevant anxiety disorders in 19.5% and 17.6% for 2015 and 2016, respectively, as compared to a prevalence of 8.4% in the controls. Riva et al. found that 20% had anxiety disorders in their 35 TTP patients [7]. Regarding iTTP, survivors depression seems to be more common than anxiety disorders [7,32]. Gender, age and partnership [33] did not seem to be related to depression in our patients, whereas comorbidities did. This is congruent with the data of Härter et al. [31]. This is important since more than two-thirds of iTTP patients suffer from at least one other disease. Long-term data from the Oklahoma TTP registry showed a significantly higher prevalence of obesity, systemic lupus erythematosus, diabetes mellitus, arterial hypertension and major depression in survivors of iTTP [8,20,30]. Depression and anxiety are associated with increased morbidity and mortality. Martin-Subero et al. demonstrated for 803 inpatients over a follow-up period of 18 years that major depression was associated with a 2.4-times higher risk of mortality [34], independent of their disease. According to Cuijpers and Smit, mortality is increased regardless of the severity of depression [17]. Given that depressive symptoms affected up to 60% of our iTTP patients, together with low quality of life scores, antidepressive therapy seems mandatory. Lewis et al. [35], Cataland et al. [5] and Riva et al. [7] also reported a significantly compromised quality of life in iTTP patients. The number and severity of survived acute episodes do not seem to have a significant influence on the development and severity of depression [10]. An abnormal cerebral MRI scan during an acute episode does not implicate an increased likelihood of the development of depression or an anxiety disorder [32]. The survey on the attitude to life and resilience of our iTTP patients suggests that the patients were less resilient and optimistic, but nevertheless, not more pessimistic than the control group. The resilience of our iTTP patients was negatively related to the severity of their depressive symptoms. This is congruent with the findings, for example, in dry eye disease or cardiovascular disease [36,37]. According to other studies, more resilient individuals develop less depression and anxiety overall, regardless of whether they have a severe underlying disease [38–41]. The resilience may be reduced by the experience of a life-threatening disease and cognitive deficits, which further increases the risk of depression in iTTP.

Limitations of the Study

Our study has limitations: First, we used self-report questionnaires. There was no examination by a clinician, such as in the studies by Han et al. [6]. However, we used questionnaires that have been widely validated in large cohorts of healthy subjects and patients. Second, only about 60% of our iTTP survivors participated in the self-evaluation study. Symptomatic patients may have been more motivated to answer the survey compared to asymptomatic patients. On the other hand, severely depressive patients may also have declined participation. The exact clinical data on the severity of the iTTP were not fully available for all patients, and comorbidities were not confirmed beyond the self-reporting. Finally, we do not have data on mental illness or resilience prior to the iTTP diagnosis.

5. Conclusions

The survivors of acute iTTP are significantly more likely to suffer from depressive and anxiety disorders as compared to the general population. The patients also reported a significantly compromised quality of life and perceived their cognitive performance as being significantly reduced. Overall, the iTTP patients were less optimistic and showed a significantly lower resilience, which in turn correlated strongly with the severity of the depression. It remains to be investigated whether psychological counseling in these long-term patients helps to improve neuropsychiatric disorders during long-term follow-ups. Furthermore, there is hope that new treatment strategies aiming at a fast resolution of the microvascular thrombotic process may improve long-term outcomes [42].

Supplementary Materials: The following are available online at https://www.mdpi.com/2077-0383/10/2/365/s1, Figure S1: Patient recruitment and response rates for the four surveys of the cohort of iTTP patients from Mainz, Table S1: Correlation of the PHQ-9 score (depressive symptoms) with sex, age, physical activity, partnership status and comorbidities in the 2015 and 2016 questionnaires.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to data privacy act.

Conflicts of Interest: The authors state that they have no conflict of interest with this publication. Inge Scharrer is a member of the Data Safety Monitoring Board in the BAX 930 study (investigating recombi-nant ADAMTS13 infusion in hereditary TTP). Bernhard Lämmle is chairman of the Data Safety Monitoring Committee of the BAXALTA 281102 and the SHP655201 studies (now both run by TAKEDA), investigating recombinant ADAMTS13 in congenital and acquired TTP, respectively. He is on the Advisory Board of Sanofi for Caplacizumab, and received travel and accommodation support for participating at scientific meetings and/or lecture fees from Ablynx, Alexion, Siemens, Bayer, Roche, and Sanofi.

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No Evidence for Classic Thrombotic Microangiopathy in COVID-19

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Abstract: Background: Coronavirus disease-2019 (COVID-19) triggers systemic infection with involvement of the respiratory tract. There are some patients developing haemostatic abnormalities during their infection with a considerably increased risk of death. Materials and Methods: Patients (n = 85) with SARS-CoV-2 infection attending the University Medical Center, Mainz, from 3 March to 15 May 2020 were retrospectively included in this study. Data regarding demography, clinical features, treatment and laboratory parameters were analyzed. Twenty patients were excluded for assessment of disseminated intravascular coagulation (DIC) and thrombotic microangiopathy (TMA) due to lack of laboratory data. Results: COVID-19 patients (n = 65) were investigated, 19 with uncomplicated, 29 with complicated, and 17 with critical course; nine (13.8%) died. Seven patients showed overt DIC according to the ISTH criteria. The fibrinogen levels dropped significantly in these patients, although not below 100 mg/dl. Hallmarks of TMA, such as thrombocytopenia and microangiopathic haemolytic anaemia, were not detected in any of our COVID-19 patients. ADAMTS13 activity was mildly to moderately reduced in 4/22 patients, all having strongly elevated procalcitonin levels. Conclusion: DIC occurred in 7/65 COVID-19 patients but fibrinogen and platelet consumption were compensated in almost all. ADAMTS13 assays excluded TTP and hallmarks of classic TMA were absent in all investigated patients. We hypothesize that the lacking erythrocyte fragmentation and only mild platelet consumption in severe COVID-19 are due to a microangiopathy predominantly localized to the alveolar microcirculation with a low blood pressure gradient.

Keywords: coronavirus disease; COVID-19; ADAMTS13; microangiopathy; disseminated intravascular coagulation

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1. Introduction

Patient age, male sex and pre-existing comorbidities are the major determinants of clinical severity and outcome of coronavirus disease-2019 (COVID-19) caused by Severe

Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) [1–3]. Some haemostasis parameters have been associated with poor outcome [4–6] and D-dimer values >2 µg/mL predicted mortality among hospitalized COVID-19 patients [7]. These findings led to the recommendation to monitor haemostasis parameters in COVID-19 patients [8]. In line with laboratory surrogates of activated coagulation, COVID-19 is associated with increased rates of thromboembolic events in 15% up to 69% [9–13]. Autopsy studies have confirmed that large vessel thromboembolic events and microthrombosis contribute to structural lung damage and respiratory failure [14–16]. Disseminated intravascular coagulation (DIC), as identified by a diagnostic DIC score, was found in 15/21 (71.4%) patients with fatal outcome but only 1/162 (0.6%) survivors [4]. DIC potentially evolves from endothelial activation or endothelial damage triggered by SARS-CoV-2 infection and subsequent consumption of plasmatic coagulation factors during COVID-19 [17–19]. DIC causes microvascular thrombosis, subsequent tissue malperfusion, and eventually drives multi organ failure. The clinical presentation of DIC during COVID-19 may have a similar appearance as thrombotic microangiopathies (TMAs) [20]. Classic TMAs, including thrombotic thrombocytopenic purpura (TTP), haemolytic uremic syndrome (HUS) and a series of other TMAs, describe an etiologically very heterogeneous group of conditions. TMAs are characterized by microvascular endothelial damage with increased release of von Willebrand factor (VWF) and widespread arteriolar and capillary thrombosis leading to the diagnostic hallmarks of consumptive thrombocytopenia and microangiopathic haemolytic anaemia (MAHA) with schistocytes in the blood smear [21,22].

A massive release of VWF from the endothelial cells, as it occurs in severe inflammatory states and systemic infections, can lead to a mild decrease of VWF-cleaving protease, a disintegrin and metalloprotease with thrombospondin type 1 motif 13 (ADAMTS13) [23]. Whether the resulting VWF/ADAMTS13 dysbalance contributes to the pathophysiology of certain TMAs in a similar way as in classic TTP characterized by a very severe deficiency of ADAMTS13 activity (<5–10% of normal) remains unclear.

We investigated clinical and laboratory patterns in this observational study to understand more about the underlying coagulopathy during COVID-19. In particular, we focused on clinical and laboratory features of DIC and classic TMAs.

2. Materials and Methods

Patients (n = 85) with confirmed SARS-CoV-2 infection who were seen at the University Medical Center, Mainz, Germany, between 3 March and 15 May 2020 were assessed in this observational study. Patient characteristics and laboratory findings were reviewed retrospectively through the electronic hospital information systems (i.s.h.med®, SAP, Weinheim Germany, Nexus Swisslab, Berlin, Germany). The retrospective study was approved by German law [Landeskrankenhausgesetz §36 and §37] in accordance with the Declaration of Helsinki and by the local Ethics Committee of "Landesärztekammer Rheinland-Pfalz" (reference numbers: 2020-14988_2).

Severity of COVID-19 was classified by respiratory function into an uncomplicated, complicated, and critical clinical course. Patients with uncomplicated disease required neither monitoring nor oxygen supplementation, whereas patients affected by complicated COVID-19 were in need for oxygen supplementation and critically ill COVID-19 patients needed invasive ventilation. The categorization into the individual COVID-19 severity stages was done retrospectively based on the clinical course during hospitalization.

SARS-CoV-2 infection was confirmed by polymerase chain reaction (PCR) from respiratory samples, employing a PCR kit specific for SARS CoV-2 (Altona Diagnostics GmbH, Hamburg, Germany). All other laboratory assays were performed in the accredited (DIN-ISO 15.189) Institute of Clinical Chemistry and Laboratory Medicine of the University Medical Center, Mainz. Renal injury was assessed based on Acute Kidney Injury Network (AKIN) criteria [24,25]. Presence of DIC was determined according to ISTH guidelines by the DIC score, incorporating platelet count, D-dimer, INR and fibrinogen level [26]. D-Dimer, derived fibrinogen and prothrombin time (PT/INR) were performed on ACL

TOP 750 instruments (Instrumentation Laboratory Company, IL, Bedford, MA, USA) using IL reagents (HemosIL D-Dimer HS 500 and HemosIL RecombiPlasTin 2G) and following the manufacturer's instructions. ADAMTS13 activity was examined by the fluorescence resonance energy transfer system (FRETS-VWF73) method [27] modified according to Kremer-Hovinga et al. [28].

Statistical analyses employed SPSS version 22.0 (IBM GmbH, Ehningen, Germany). Descriptive statistics included frequency, mean, standard deviation, median, interquartile range (IQR), minimum and maximum. Explorative group comparisons were performed by *t*-test or Mann–Whitney-U-test for continuous variables and by Chi-squared test or Fisher's exact test for categorical variables, accordingly. *p*-values are two tailed, and p values < 0.05 were considered statistically significant.

Sixty-three/65 (97%) COVID-19 patients were hospitalized and 20/65 (31%) had to be treated in intensive care unit. COVID-19 patients were predominantly male (63%) and had a median age of 69 (IQR 57–79, range 22–86) years. Older adults (age > 65 years) accounted for 37/65 (57%). The most common underlying comorbidities were arterial hypertension, cardiovascular disease and diabetes mellitus. Obesity (BMI $\geq$ 30 kg/m^2) was observed in 23/62 (37%) and a history of venous thromboembolic events before COVID-19 in 4/65 (6%). The clinical course of COVID-19 was uncomplicated in 19 (29%), complicated in 29 (45%) and critical in 17 (26%). The overall mortality rate was 14% (9/65), reaching 35% (6/17) in critical COVID-19. Invasive ventilation of 17 critically ill patients was performed over a period of 21 (median, IQR 7–30) days. Prophylactic or therapeutic anticoagulation was used in 43/46 (94%) patients with complicated or critical COVID-19 manifestation and in 14/19 (74%) of the uncomplicated cases (Table 1).

3. Results

3.1. Patient Characteristics

A total of 85 patients with proven SARS-CoV-2 infection were seen between 3 March and 15 May 2020. For the assessment of haemostatic alterations, 20 patients were excluded due to insufficient laboratory data (Table 1 and Table S1.1).

Table 1. Characteristics of 65 patients with COVID-19 and analyzed for haemostatic abnormalities.

Characteristics	Total	Uncomplicated COVID-19	Complicated COVID-19	Critical COVID-19	DIC [a]
Number	65	19	29	17	7
Age (years) (IQR)	69 (57–79)	64 (39–79)	74 (60–81)	66 (53–73)	76 (55–80)
Sex (male/female)	41/24 (63.1/36.9%)	10/9 (52.6/47.4%)	17/12 (58.6/41.4%)	14/3 (82.4/17.6%)	7/0 (100/0.0%)
BMI (kg/m^2) (IQR) [b]	27.2 (24.2–33.6)	26.7 (22.5–32.6)	29.4 (24.4–33.6)	27.0 (25.7–33.5)	27.0 (24.7–29.4)
Preexisting comorbidities					
Arterial Hypertension	38 (58.5%)	9 (47.4%)	9 (31%)	10 (58.8%)	6 (85.6%)
Diabetes mellitus	14 (21.5%)	1 (5.3%)	8 (27.6%)	5 (29.4%)	2 (28.6%)
Obesity (BMI $\geq$30 kg/m^2) [b]	23/62 (37.1%)	5/16 (31.1%)	13/29 (44.8%)	5/17 (29.4%)	1/7 (14.3%)
Chronic respiratory disease	11 (16.9%)	2 (10.5%)	6 (20.7%)	3 (17.6%)	1 (14.3%)
Cardiovascular disease	17 (26.2%)	5 (26.3%)	8 (27.6%)	4 (23.5%)	3 (42.9%)
Cerebrovascular disease	11 (16.9%)	2 (10.5%)	3 (10.3%)	6 (35.3%)	1 (14.3%)
Terminal renal insufficiency	2 (3.1%)	1 (5.3%)	1 (3.4%)	0 (0.0%)	0 (0.0%)
Venous thromboembolic history	4 (6.2%)	0 (0.0%)	1 (3.4%)	3 (17.6%)	1 (14.3%)
Pulmonary Embolism	1 (1.5%)	0 (0.0%)	1 (3.4%)	0 (0.0%)	0 (0.0%)
Deep vein thrombosis	4 (6.2%)	0 (0.0%)	1 (3.4%)	3 (17.6%)	1 (14.3%)
Preexisting anticoagulation					
DOAC	1 (1.5%)	0 (0.0%)	1 (3.4%)	0 (0.0%)	1(14.3%)
Vitamin K antagonist	1 (1.5%)	0 (0.0%)	0 (0.0%)	1 (5.9%)	0 (0.0%)
Platelet aggregation inhibitors	19 (29.2%)	8 (42.1%)	7 (24.1%)	4 (23.5%)	0 (0.0%)

Table 1. *Cont.*

Characteristics	Total	Uncomplicated COVID-19	Complicated COVID-19	Critical COVID-19	DIC [a]
Clinical complications during COVID-19 infection					
Renal failure	18 (27.7%)	0 (0.0%)	5 (17.2%)	13 (76.5%)	4 (57.1%)
AKIN 1	3 (4.6%)	0 (0.0%)	2 (6.9%)	1 (5.9%)	0 (0.0%)
AKIN 3	15 (23.1%)	0 (0.0%)	3 (10.3%)	12 (70.6%)	4 (57.1%)
Thromboembolic events	4 (6.2%)	1 (5.3%)	0 (0.0%)	3 (17.6%)	1 (14.3%)
Acute coronary syndrome	3 (4.6%)	0 (0.0%)	2 (6.9%)	1 (5.9%)	1 (14.3%)
Myocarditis	2 (3.1%)	2 (3.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Medical care during COVID-19 infection					
Hospitalized	63 (96.9%)	17 (89.5%)	29 (100%)	17 (100%)	7 (100%)
Intensive care	20 (30.8%)	0 (0.0%)	3 (10.3%)	17 (100%)	4 (57.1%)
Oxygen supplementation	46 (70.8%)	-	29 (100%)	17 (100%)	7 (100%)
Invasive ventilation	17 (26.2%)	-	-	17 (100%)	4 (57.1%)
Renal replacement therapy	10 (15.4%)	0 (0.0%)	0 (0.0%)	10 (58.8%)	3 (42.9%)
Anticoagulation during COVID-19 infection					
None	7 (10.8%)	5 (26.3%)	2 (6.9%)	0 (0.0%)	1 (14.3%)
Prophylactic dose LMWH	43 (66.2%)	13 (68.4%)	25 (86.2%)	5 (29.4%)	2 (28.6%)
Therapeutic dose LMWH	14 (21.5%)	1 (5.3%)	1 (3.4%)	12 (70.6%)	3 (42.9%)
DOAC	1 (1.5%)	0 (0.0%)	1 (3.4%)	0 (0.0%)	1 (14.3%)
Clinical outcome of COVID-19 infection					
Uncomplicated	19 (29.2%)	19 (100%)	-	-	0 (0.0%)
Complicated	29 (44.6%)	-	29 (100%)	-	3 (42.9%)
Critical	17 (26.2%)	-	-	17 (100%)	4 (57.1%)
Deceased	9 (13.8%)	0 (0%)	3 (10.3%)	6 (35.3%)	3 (42.9%)

Patient characteristics are presented as median (interquartile range) or number (%). Explorative comparisons of patient subgroups and corresponding *p*-values are provided in supplemental Table S1.2. AKIN, AKIN Classification for Acute Kidney Injury; BMI, body mass index; COVID-19, coronavirus disease-2019; DOAC, direct oral anticoagulants; DIC, disseminated intravascular coagulation; IQR, interquartile range; LMWH, low molecular weight heparin. [a] Patients with DIC are a subset of patients with complicated and critical COVID-19. [b] BMI and obesity could only be determined in 62 of 65 patients due to missing anthropometric data.

3.2. COVID-19-Associated Laboratory Parameters and Organ Damage

Elevated creatinine values were observed in 38/64 (59%) throughout the course of COVID-19 (Table 2 and Table S2.1 and Figure S1). According to the Classification for Acute Kidney Injury (AKIN) patients with COVID-19 developed a new onset of renal injury (AKIN 1) and renal failure (AKIN 3) in 33/65 (5%) and 15/65 (23%), respectively (Table 1). Renal replacement therapy was eventually initiated in 10/65 (15.4%) patients (Table 1).

Elevated troponin I (>24 pg/mL) indicative of myocardial injury was observed in 27/61 (44%) of all COVID-19 patients (Table 2 and Table S2.1 and Figure S1). In patients with myocardial injury, 9/27 (33%) had arrhythmic events, 2/27 had a myocarditis and 3/27 (11%) were diagnosed with acute coronary syndrome (Table 1).

Significant increases (>5 times the upper limit of normal) in AST and ALT were observed in 17/63 (27%) and 9/64 (14%), respectively. Impaired liver function as indicated by hyperbilirubinemia (total bilirubin >1.2 mg/dL) and pronounced hypoalbuminemia (serum albumin <28 g/L) occurred in 17/64 (27%) and 31/58 (53%), respectively (Table 2 and Table S2.1 and Figure S1).

Table 2. Laboratory analyses of 65 patients with COVID-19 analyzed for haemostatic abnormalities.

Parameter	Total		Uncomplicated COVID-19		Complicated COVID-19		Critical COVID-19		DIC [a]	
Number of Patients	**65**	#	**19**	#	**29**	#	**17**	#	**7**	#
LDH^{max} (U/L)	507 (381–705)	63	421 (345–476)	17	495 (367–593)	29	705 (629–786)	17	832 (641–1849)	7
AST^{max} (U/L)	76 (46–187)	63	49 (35–76)	19	69 (47–110)	28	206 (103–394)	16	188 (93–229)	6

Table 2. *Cont.*

Parameter	Total		Uncomplicated COVID-19		Complicated COVID-19		Critical COVID-19		DIC [a]	
Number of Patients	**65**	#	**19**	#	**29**	#	**17**	#	**7**	#
ALT^{max} (U/L)	51 (33–1429)	64	41 (31–66)	18	43 (28–69)	29	156 (75–395)	17	111 (22–927)	7
GGT^{max} (U/L)	87 (43–180)	63	58 (39–140)	18	66 (3–113)	28	526 (145–988)	17	149 (44–988)	7
Total bilirubin^{max} (mg/dl)	0.8 (0.6–1.4)	64	0.7 (0.5–0.8)	18	0.7 (0.6–0.9)	29	2.5 (1.4–3.3)	17	2.8 (1.5–7.0)	7
Albumin^{min} (g/L)	26 (20–32)	58	30 (25–35)	17	28 (24–33)	28	12 (11–16)	13	16 (10–22)	7
CK^{max} (U/L)	301 (97–798)	62	161 (76–303)	19	226 (77–490)	26	1359 (768–2616)	17	480 (154–2357)	7
TNI^{max} (pg/mL)	18.7 (10.6–89.3)	61	16.8 (5.8–56.5)	19	15.8 (6.6–21.2)	25	89.3 (35.3–421)	17	70.7 (15.8–421)	7
Creatinine^{max} (mg/dL)	1.2 (0.93–2.1)	64	0.96 (0.74–1.18)	19	1.21 (0.90–1.64)	28	2.16 (1.64–3.33)	17	2.16 (1.56–4.35)	7
Hemoglobin^{min} (g/dL)	10.3 (8.0–12.5)	65	11.8 (7.6–13.6)	19	11.5 (10.3–12.6)	29	7.4 (7.0–8.4)	17	8.0 (6.7–11.4)	7
Platelet count^{min}/nL	178 (134–227)	65	184 (134–233)	19	185 (142–233)	29	170 (133–183)	17	71 (49–160)	7
Absolute leukocytes^{max}/nL	7.6 (5.4–9.7)	62	6.72 (4.6–8.3)	18	7.0 (5.6–9.5)	29	9.2 (7.3–11.4)	15	8.0 (6.0–17.3)	7
Absolute neutrophiles^{max}/nL	5.7 (3.5–8.7)	62	4.5 (3.0–6.9)	18	5.4 (3.9–7.8)	29	8.1 (5.1–10.0)	15	5.8 (4.6–13.8)	7
Absolute lymphocytes^{min}/nL	0.7 (0.5–1.0)	62	0.9 (0.6–1.4)	18	0.6 (0.5–1.0)	29	0.6 (0.5–0.8)	15	0.6 (0.4–0.9)	7
CRP^{max} (mg/lL)	172 (83–285)	65	118 (39–176)	19	121 (48–184)	29	385 (348–413)	17	348 (176–458)	7
PCT^{max} (ng/mL)	0.18 (0.05–1.20)	65	0.05 (0.02–0.24)	19	0.08 (0.05–0.24)	29	4.30 (1.0–11.0)	17	7.00.(0.77–17.0)	7
INR^{max}	1.2 (1.1–1.5)	65	1.2 (1.1–1.3)	19	1.2 (1.1–1.3)	29	1.6 (1.5–2.2)	17	2.6 (1.5–3.6)	7
Fibrinogen^{max} (mg/dL)	586 (476–759)	58	518 (479–582)	16	495 (442–610)	25	855 (730–897)	17	586 (517–890)	7
Fibrinogen^{min} (mg/dL)	465 (358–531)	58	428 (287–505)	16	474 (389–561)	25	469 (364–531)	17	371 (284–453)	7
D-dimer^{max} (mg/L)	1.67 (0.86–5.08)	65	1.12 (0.51–1.93)	19	1.14 (0.69–2.27)	29	6.16 (4.36–17.23)	17	7.07 (2.24–40.24)	7

Maximum (max) or minimum (min) laboratory values of each patient during the follow up until 15 May 2020 presented as medians (interquartile ranges). Explorative comparisons of patient subgroups and corresponding *p*-values are provided in Supplementary Table S2.2. CK, creatine kinase; COVID-19, coronavirus disease-2019; CRP, C-reactive protein; DIC, disseminated intravascular coagulation; AST, aspartate-aminotransferase, ALT, alanine-aminotransferase; GGT, gamma-glutamyltransferase; INR, international normalized ratio; LDH, lactate dehydrogenase; PCT, procalcitonin; TNI, troponin I. [#] Number of total patients and patients with uncomplicated, complicated, and critical COVID-19 course, and with DIC for whom laboratory values were available. [a] Patients with DIC are a subset of patients with complicated and critical COVID-19.

3.3. COVID-19 Associated Haemostatic Alterations

3.3.1. Thromboembolic Events during COVID-19

Among our COVID-19 patients, acute new-onset thromboembolic (TE) events were observed in 4/65 (6%) (Table 1). These TE events predominantly affected patients with critical COVID-19 (3/17, 18%) and only 1/19 patients (5%) with uncomplicated COVID-19. The TE events during critical COVID-19 included two patients with venous thrombosis associated with central vein catheters and one with acute arterial mesenterial infarction. The patient with uncomplicated COVID-19 developed a segmental pulmonary embolism. Twenty-seven patients had cardiac troponin I elevation (Table S2.1) and three of them required interventional coronary angioplasty for acute coronary syndrome (Table 1).

As shown in Table 1, 57/65 patients (88%) were treated with prophylactic or therapeutic doses of LMW-heparin from the time of hospitalization. In particular, all patients who developed thromboembolic events during COVID-19 received therapeutic doses of

LMW-heparin. One patient had been under rivaroxaban because of deep vein thrombosis and lung embolism that had occurred prior to COVID-19. No major bleeding event was observed during hospitalization and heparin-induced thrombocytopenia did not occur.

3.3.2. D-Dimers, Fibrinogen, INR, Platelet Count

Elevation of INR and D-dimers along with COVID-19 severity was evident (Table 2 and Table S2.1 and Figure 1). Consequently, the highest median INR (1.6, IQR 1.5–2.2, range 1.4–10.6) and highest median D-dimer (6.16 mg/L, IQR 4.36–17.23 mg/L, range 1.22–43.72 mg/L) was reached among critically ill COVID-19 patients. D-dimer concentrations > 2 mg/L, which had been associated with adverse outcome [7], were observed in 25/65 (40%) of the entire cohort and in 13/17 (81%) patients with critical COVID-19 (Table 2). In contrast, platelet counts and fibrinogen levels did not significantly differ between patient subgroups of increasing COVID-19 severity (Table 2 and Table S2.1 and Figure 1).

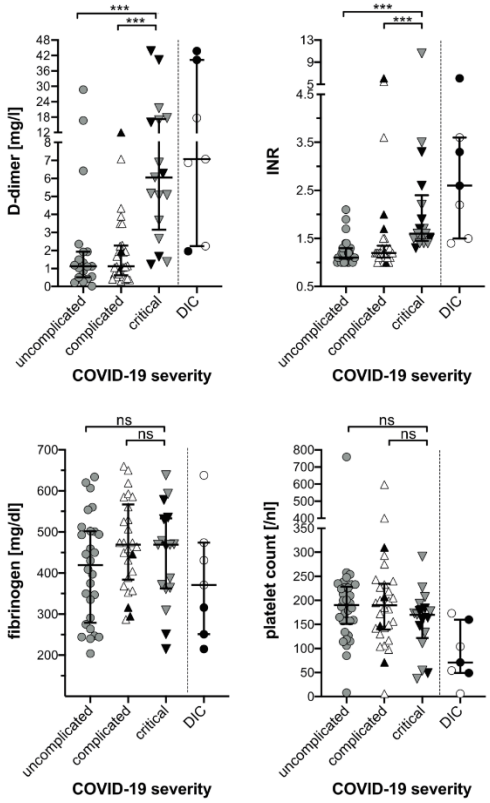

Figure 1. Haemostasis parameters of COVID-19 patients. The highest values for D-dimer and INR as well as the lowest values for fibrinogen and platelet count are plotted against the severity of COVID-19 disease and the occurrence of DIC as indicated. Black symbols represent laboratory values of deceased COVID-19 patients. Median and interquartile range are provided. Comparisons between subgroups were based on Mann–Whitney U Test (ns, not significant; *** $p < 0.001$). COVID-19, coronavirus disease-19; DIC, disseminated intravascular coagulation; INR, international normalized ratio.

Fibrinogen levels in COVID-19 patients were elevated in 49/58 (84%) at baseline (median 531, IQR 465–660, range 275–933 mg/dL). Particularly all patients (17/17) with critical COVID-19 presented with elevated baseline fibrinogen levels (median 714, IQR 613–855, range 466–933 mg/dL). During the course of COVID-19 maximum fibrinogen concentrations were above normal in the entire COVID-19 cohort (Table 2 and Table S2.1) and remained elevated for several days in most patients

3.3.3. Patients with DIC

Overt disseminated intravascular coagulation (DIC), as defined by a DIC Score ≥5 points (26), was present in 7/65 (11%) patients (Table 3 and Table S3, Figure 2).

Table 3. Constituting subscores of the DIC score in the seven patients with overt DIC.

Parameters of DIC Score		Points	Number of Patients (%)
Meeting the ISTH Criteria of Overt DIC (total points ≥5) [a]			7 (100)
Platelets	>100/nL	0	3 (42.8)
	50–100/nL	1	2 (28.6)
	<50/nL	2	2 (28.6)
D-dimer	normal (<0.5 mg/L)	0	0 (0)
	moderately elevated (0.5–2.0 mg/L)	2	1 (14.3)
	extremely elevated (>2.0 mg/L)	3	6 (85.7)
INR	<1.25	0	0 (0)
	1.25–1.7	1	2 (28.6)
	>1.7	2	5 (71.4)
Fibrinogen	≥100 mg/dL	0	7 (100)
	<100 mg/dL	1	0 (0)

[a] ISTH-DIC score according to Taylor et al., 2001 [26]; DIC, disseminated intravascular coagulation; INR, international normalized ratio; ISTH, International Society of Thrombosis and Haemostasis.

The dynamics of laboratory values included into the DIC score are shown for the seven patients who developed DIC over the course of hospitalisation (Figure 2). The DIC score became positive after a median of 21 (range 6.5–40) days post hospital admission. DIC diagnosis was mainly based on moderately (0.5–2.0 mg/L) or extremely (>2.0 mg/L) elevated D-dimers and an INR above 1.7. Patients with positive DIC score included only four patients (Pat.ID 24/ 50/ 74/ 79) with thrombocytopenia (platelets < 100/nL) (Figure 2). Fibrinogen levels were elevated in all seven patients at hospitalisation, followed by a decrease in fibrinogen levels over time (Figure 2). However, fibrinogen remained ≥100 mg/dl in all seven patients meeting the criteria for DIC (Table 3 and Figure 2). DIC was only diagnosed in male patients with complicated or critical COVID-19, three patients died (Table 1 and Figure 2). DIC patients had more pronounced LDH, total bilirubin and creatinine elevations as compared to the non-DIC group (Table 2 and Table S2.2). Two patients received low molecular weight heparin in prophylactic doses, three patients in therapeutic doses and one patient continued rivaroxaban introduced prior to COVID-19 infection (Table 1).

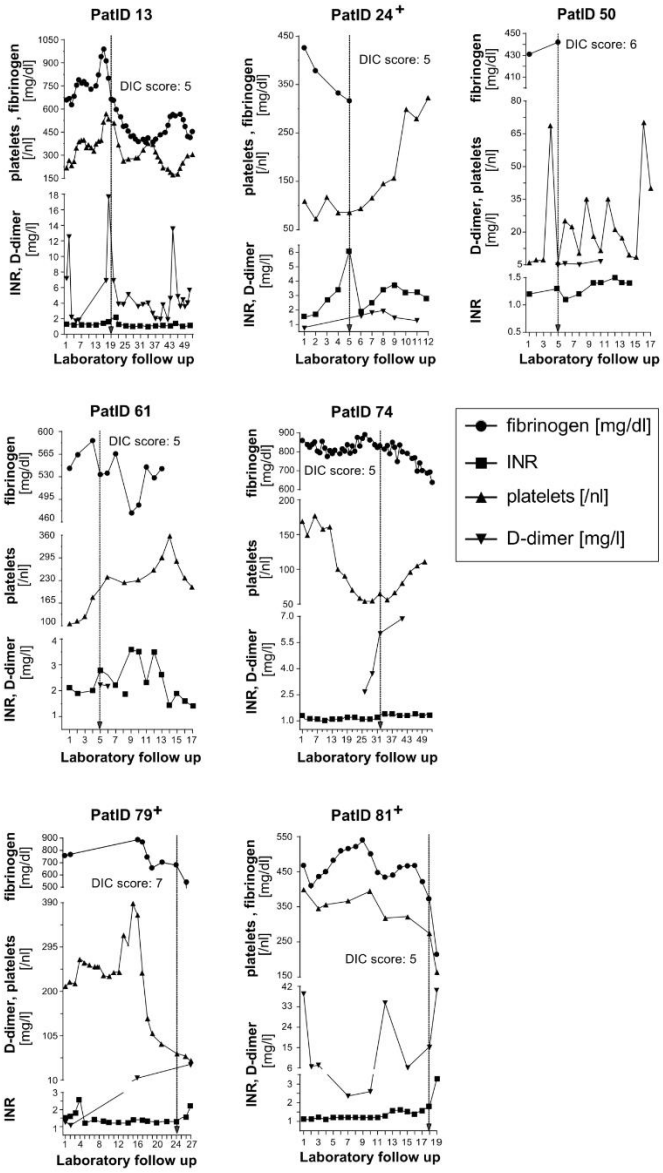

Figure 2. Time course of fibrinogen, INR, platelet count and D-dimer values in seven patients who developed DIC during COVID-19. The engraved arrow indicates the time of DIC manifestation as defined by the corresponding DIC score. Three patients with DIC died as indicated (+). DIC, disseminated intravascular coagulation; INR, international normalized ratio; PatID, patient identification number.

3.3.4. Markers of Endothelial Damage and Thrombotic Microangiopathy

Some authors reported that severe COVID-19 may cause thrombotic microangiopathy (TMA) [20,29–31]. Therefore, we examined patients for microangiopathic haemolytic anaemia (MAHA) and consumptive thrombocytopenia as hallmarks of classic TMA [21].

Anaemia using a haemoglobin cut-off for males (<13.5 g/dL) and females (<12.0 g/dL) was observed in 52/65 (80%) of our COVID-19 patients (Table S2.1). Haemoglobin reduction paralleled the severity of COVID-19, leading to anaemia in 23/29 (79%) patients with complicated COVID-19 and in 17/17 (100%) patients with critical COVID-19. Despite elevated LDH levels in 60/63 (95%) patients (Table S2.1) being compatible with haemolysis, available haptoglobin values from 22 patients across all COVID-19 stages were all normal or elevated (Table 4). In addition, schistocytes were either absent in the peripheral blood smear of 20 investigated patients or only minimally elevated (i.e., 5‰ and 9‰) in two. Based on these findings MAHA with intravascular haemolysis was not observed in this cohort (Table 4).

Platelet counts were <150/nL in 22/65 (34%) without clear relation to disease severity (Table S2.1). However, thrombocytopenia was generally mild (platelets 100 – <150/nL) in 15/65 (23%) or moderate (platelets 50 – <100/nL) in 4/65 (6%) (Table S2.1). Severe thrombocytopenia (platelets <50/nL) was found in only three (5%) patients of whom one had received cytotoxic chemotherapy.

Finally, ADAMTS13 was mostly in the normal range and only 4/22 tested showed reduced ADAMTS13 activity values (<50%) with a minimum ADAMTS13 activity of 17.8% (Table 4). These mild or moderately decreased ADAMTS13 activity values excluded thrombotic thrombocytopenic purpura in any patient. Most prominent finding in these 22 patients, comprehensively tested for the presence of TMA, were the elevated VWF activity (median 329%, IQR 195 – >390%) and antigen (median 232%, IQR 219–498%) levels (Table 4). The VWF antigen/ ADAMTS13 activity ratio was elevated in 21/21 (100%) patients tested (median 3.4, IQR 2.6–7.7, range 2.1–33.4) (Table 4).

It is remarkable that in the four patients with mild to moderately reduced ADAMTS13 activity (18–48%) by far the highest procalcitonin (PCT) values were found. Bacterial coinfections were confirmed in two cases by *Escherichia coli* and *Staph epidermidis* isolates from the lower respiratory system and blood stream, respectively.

In sum, there were no diagnostic clues for classic TMA (lacking schistocytes, normal or elevated haptoglobin levels, no severe thrombocytopenia) in any of our patients whereas the consistently high VWF levels were compatible with an endothelial activation and/or damage.

Table 4. Thrombotic microangiopathy-related factors of 22 comprehensively tested COVID-19 patients. All laboratory parameters were determined on the same day, except for the lowest platelet count.

PatID	Timepoint [a]	Schistocytes [‰]	Haptoglobin [g/L]	Platelet Count [/nL]	Lowest [b] Platelet Count [/nL]	VWF Activity [%]	VWF Antigen [%]	ADAMTS13 Activity [%]	Ratio VWF:AG/ ADAMTS13 Act	PCT [ng/mL]	CRP [mg/L]	Severity of COVID-19
normal	**Days**	**<5**	**0.14–2.73**	**150–360**	**150–360**	**40–170**	**42–176**	**≥ 50**	**0.5–2.0 [c]**	**<0.5**	**<5.0**	
13	**24**	**3**	**0.22**	**309**	**173**	**n.m.**	**n.m.**	**48.0**		**1.1**	**89**	**cr (+DIC)**
29	2	Negative	2.22	208	184	170	209	64.0	3.3	0.03	7.3	uc
36	1	Negative	4.15	293	293	344	396	55.8	7.1	0.15	151	co
40	2	Negative	2.96	341	179	>390	654	75.4	8.7	0.06	31	uc
41	1	Negative	1.52	437	401	170	218	75.1	2.9	0.03	26	co
44	**0**	**1**	**2.55**	**85**	**85**	**>390**	**511**	**31.2**	**16.4**	**3.9**	**254**	**uc**
44	**1**	**5**	**2.44**	**106**	**85**	**>390**	**458**	**40.7**	**11.3**	**2.9**	**156**	**uc**
45	1	Negative	3.33	165	165	246	253	64.3	3.9	0.4	204	uc
48	2	Negative	4.25	305	275	328	409	71.6	5.7	0.12	186	co
52	1	Negative	3.51	682	597	158	218	63.5	3.4	0.04	37	co
55	14	Negative	2.96	224	193	329	221	85.4	2.6	0.12	46	cr
56	1	Negative	1.76	227	227	188	199	85.4	2.3	0.03	109	uc
58	**1**	**Negative**	**1.79**	**250**	**200**	**>390**	**782**	**46.0**	**17.0**	**3.5**	**184**	**co**
59	0	9	3.31	240	213	336	226	88.5	2.6	0.02	32	uc
70	9	Negative	1.44	392	187	215	228	80.0	2.9	0.05	15	uc
73	0	Negative	1.71	254	254	178	202	97.8	2.1	<0.02	0.79	uc
74	**44**	**Negative**	**2.52**	**54**	**54**	**>390**	**595**	**17.8**	**33.4**	**6.8**	**325**	**cr (+DIC)**
77	15	Negative	3.81	245	119	>390	613	90.9	6.7	0.12	32	uc
78	1	Negative	3.51	186	159	361	222	85.4	2.6	0.23	179	uc
79+	1	Negative	5.45	220	49	>390	602	76.4	7.9	0.31	155	cr (+DIC)
81+	0	n. m.	n.m.	345	160	267	234	87.0	2.7	0.28	169	cr (+DIC)
84	0	Negative	4.21	759	759	263	231	71.8	3.2	0.24	138	uc
85	1	Negative	3.30	238	238	162	195	84.8	2.3	0.03	39	Uc

Data of patients with decreased ADAMTS13 activity are in bold. CRP, C-reactive protein; COVID-19, coronavirus disease-19; DIC, disseminated intravascular coagulation; PatID, patient identification number; PCT, procalcitonin; uc, uncomplicated; co, complicated; cr, critical COVID-19 course; n. m., not measured; VWF:AG, von Willebrand-factor antigen. [a] Day when TMA-related factors were assayed after hospitalisation. [b] Lowest platelet count of each patient during hospitalization. [c] In healthy normal controls the ratio of VWF:Ag/ADAMTS13 act is usually between about 0.5 and 2.0, **+** Patients who died from COVID-19 during hospitalization.

4. Discussion

We present here our single-centre cohort of all consecutive, retrospectively included patients diagnosed with COVID-19 at the University Medical Center Mainz during the first wave of the pandemic from 3 March to 15 May 2020. Our report focuses on the clinical and laboratory abnormalities related to thrombosis and haemostasis. Multiple scientific publications pointing to the high thrombotic risk in COVID-19 [9–13,32], trying to understand its pathophysiology [33–35], suggesting the prognostic value of haemostatic laboratory parameters [4,36,37] and proposing prophylactic and/or therapeutic measures to improve the outcome have been provided [11,38].

From 65 of the 85 registered COVID-19 patients sufficient laboratory data were available to delineate the frequency and extent of haemostatic abnormalities (Table 1). Nine of the patients died, 3 of 29 with complicated and 6 of 17 with critical disease. No autopsies were performed. The incidence of clinically manifest thromboembolic events was rather low and included three venous TE, one mesenteric arterial infarction and three acute coronary syndromes needing percutaneous coronary intervention (Table 1), which is substantially lower than reported by several authors [12,13]. Whereas initially published cohorts from China had not received TE prophylaxis [9], other series of patients had a cumulative incidence of TE events up to about 15–60% (dependent on COVID-19 severity and length of hospitalisation) despite prophylactic or even higher-than-prophylactic doses of LMWH given [13]. Major bleeding events in patients with or without anticoagulation were generally rare [39].

Routine haemostatic laboratory parameters showed strongly elevated fibrinogen and D-dimer levels, the latter being exceedingly high in those with critical disease (Table 2, Figure 1). Prothrombin times expressed as INR were mildly elevated in critical patients and platelet counts were mostly normal and sometimes subnormal independent of COVID-19 severity. Calculating the DIC score [26] taking into account the highest D-dimer and INR and the lowest fibrinogen and platelet count values showed seven of 65 patients having a score $\geq$5 signalling overt DIC (Table 3 and Table S3). The course of laboratory parameters in these seven individual patients shows a notable difference of their "DIC pattern" as compared to "typical DIC" associated with bacterial sepsis, obstetric complications and other inflammatory conditions [40,41]. Six of our DIC patients showed a mild drop of fibrinogen (but none <100 mg/dL), platelet count fell below 50/nL in only two, and none of the seven showed any abnormal bleeding tendency. These data resemble observations by other groups [20,37] and new designations for the COVID-19 associated haemostatic disturbances have been proposed, i.e., "COVID-19 associated coagulopathy (CAC)" (18) or "Pulmonary intravascular coagulopathy (PIC)" [42] to stress the discrepancy to "classic DIC" [43]. Experts in this field have discussed the current evidence and suggested that CAC during severe COVD-19 should be considered as a prothrombotic phenotype of DIC [44].

Other investigators have labelled the coagulopathy in COVID-19 as thrombotic microangiopathy (TMA) [30,31,45–48]. Nevertheless, the hallmarks of classic TMA, consumptive thrombocytopenia and microangiopathic haemolysis with erythrocyte fragmentation resulting in schistocytes in the peripheral blood smear [21], have neither been found in any of our 22 patients subjected to detailed investigation for TMA (Table 4) nor in most studies from other investigators [49,50]. Mildly or moderately decreased ADAMTS13 activity (18–48% of normal in 4 of the 22 patients, the other 18 displaying normal activity >50%) (Table 4) as well as normal [50] or subnormal to normal levels [30,45,49] in several previously described patients clearly ruled out thrombotic thrombocytopenic purpura (TTP), which is characterized by <10% (and indeed often <1%) ADAMTS13 activity [51]. Most notable findings in the 22 patients were the highly elevated values of VWF activity and antigen and the increased ratio of VWF:antigen/ADAMTS13 activity (Table 4). Whether this imbalance between high VWF concentrations and low levels of its size-regulating protease in our patients, features that have also been described in patients with severe sepsis or septic shock [23,52], is pathophysiologically relevant in COVID-19 remains questionable,

especially in the absence of consumptive thrombocytopenia. Henry et al. reported that a decreasing ratio of ADAMTS13:act/ VWF:Ag in 52 COVID-19 patients at presentation to the emergency room was predictive for the development of acute kidney injury and a severe form of COVID-19 [48]. However, the decreased ratio of ADAMTS13:act/VWF:Ag in 12 of 52 patients, which did not correlate with platelet count, was mainly due to high VWF:Ag levels. This may, in turn, primarily reflect an augmented VWF release caused by endothelial injury [48]. It is well possible that the highly elevated FVIII:C levels, paralleling the increased VWF values in COVID-19 [49,50] are equally or more relevant in mediating a prothrombotic effect. The explanation for the consistently and often massively elevated VWF values is likely explained by endothelial activation and damage [14,17,50], the latter caused by direct endothelial invasion by the SARS-CoV-2 [14,53,54]. The autopsy study by Ackermann and colleagues comparing the lungs of seven patients who died from COVID-19 with those of seven who died from acute respiratory distress syndrome caused by influenza A infection and 10 age-matched control lungs showed distinctive morphologic vascular features in COVID-19. These findings included severe endothelial injury, widespread alveolar capillary fibrinous microthrombi (9-times more prevalent than in influenza-infected lungs) and marked features of (mainly intussusceptive) angiogenesis with formation of new vessels growing into the lumen of existing vessels [14]. Beigee et al. described diffuse alveolar damage and thrombotic microangiopathies in lung biopsies of 31 patients who had died from COVID-19 [16]. Moreover, other autopsy studies confirmed extensive fibrinous microthrombi in the lungs [53,55] and also in some skin lesions [53], from patients succumbed to severe COVID-19. The latter study demonstrated, in addition, significant microvascular deposition of complement activation products, C5b-9, C4d and mannose binding lectin-associated serine protease 2, in colocalization with SARS-Cov-2, hinting at systemic activation of the alternative and lectin-mediated complement pathways [53]. Thus, these studies provide unequivocal proof of "thrombotic microangiopathy", mainly in the lungs. A study of 50 children with COVID-19 including 18 with a multisystem inflammatory syndrome in children (MIS-C) suggested the presence of TMA in 17 of 19 with complete laboratory evaluation. Many showed evidence of systemic complement activation, i.e., increased sC5b-9 levels [47]. Furthermore, first studies describe the successful use of complement inhibitors in COVID-19 patients [56].

Whether the (almost uniformly) lacking laboratory hallmarks of TMA in our COVID-19 patients and those described in other cohorts [49,50] is explained by the predominantly pulmonary microangiopathy in COVID-19 remains to be investigated. While both VWF-mediated platelet adhesion and aggregation mainly in the microcirculation of brain, kidney and heart as seen in TTP [51] and fibrin microthrombi located predominantly in the kidney as seen in HUS [21,22], will result in erythrocyte fragmentation in the partially occluded microcirculation with the high blood pressure gradient in the arterial circulation, it may be hypothesized that the "COVID-19-associated thrombotic microangiopathy that is often restricted to the pulmonary microcirculation will not produce schistocytes owing to the much lower blood pressure gradient".

Limitations of the Study

Our study has several limitations. Due to the retrospective approach, relevant parameters were not monitored throughout the course of COVID-19. In particular, we did not assess markers of complement activation, which may play a pathophysiologic role in the prothrombotic alterations of COVID-19 [53] and inhibition of complement activation may be targeted therapeutically [56]. In addition, several other tests, such as viscoelastic methods to study clot formation and fibrinolysis were not performed in this COVID-19 cohort. Whether these latter methods could be useful to identify the thrombotic risk in individual patients and/or to guide antithrombotic treatment remains to be further investigated [57].

Another limitation is a bias regarding the severity of COVID-19. Mostly severely symptomatic patients presented at our University Medical Center and were admitted

as inpatients and laboratory data on haemostatic alterations were not available from all patients. Therefore, our observational study is merely hypothesis-generating.

5. Conclusions

In sum, our observational data on the haemostaseologic abnormalities in real world adult COVID-19 patients admitted to a single academic centre add results suggesting that the haemostatic alterations in severe COVID-19 are not fully fitting into the established categories of DIC and classic TMA. Further pathophysiologic research is needed to provide clinically useful targets for intervention to improve patient outcome.

Supplementary Materials: The following are available online at https://www.mdpi.com/2077-0383/10/4/671/s1, Figure S1: Organ damage markers in patients with COVID-19. Table S1.1: Comparison of the 65 patients positive for SARS-CoV-2 included (Table 1), and the 20 excluded for analysis of haemostatic alterations because of lacking laboratory data, Table S1.2: Comparison of patient characteristics between groups of varying COVID-19 severity, Table S2.1: Rates of abnormal laboratory values of 65 patients with COVID-19 and analysed for haemostatic abnormalities, Table S2.2: Comparison of laboratory parameters between groups of COVID-19 severity, Table S3: Detailed laboratory data of the seven COVID-19 patients with overt DIC (DIC score $\geq$ 5). + Patients who died from COVID-19 during hospitalization.

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7.6. Originalarbeit VI.

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REGULAR ARTICLE

blood advances

A novel von Willebrand factor multimer ratio as marker of disease activity in thrombotic thrombocytopenic purpura

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Key Points

- Eighty-three patients with iTTP were prospectively evaluated for ADAMTS13 activity, conformation, and for a novel VWF MM ratio.
- This ratio of high- to low-molecular weight VWF MM is significantly higher before an iTTP bout than in patients in continued remission.

Immune-mediated thrombotic thrombocytopenic purpura (iTTP), an autoantibody-mediated severe ADAMTS13 deficiency, is caused by insufficient proteolytic processing of von Willebrand factor (VWF) multimers (MMs) and microvascular thrombi. Recurrence of acute iTTP is associated with persistence or reappearance of ADAMTS13 deficiency. Some patients remain in remission despite recurring or persisting severe ADAMTS13 deficiency. In a prospective 2-year observational study, we investigated VWF MM patterns and ADAMTS13 in patients with iTTP in remission and at acute episodes. Of the 83 patients with iTTP, 16 suffered 22 acute episodes whereas 67 remained in clinical remission during follow-up, including 13 with ADAMTS13 <10% and 54 with ADAMTS13 ≥10%. High -molecular weight to low-molecular weight VWF MM ratio based on sodium dodecyl sulfate-agarose gel electrophoresis was compared with ADAMTS13 activity. VWF MM ratio was significantly higher in patients in remission with <10% compared with ≥10% ADAMTS13 activity. Fourteen samples obtained from 13 to 50 days (interquartile range; median, 39) before acute iTTP onset (ADAMTS13 <10% in 9 patients and 10%-26% in 5) showed VWF MM ratios significantly higher than those from 13 patients remaining in remission with ADAMTS13 <10%. At acute iTTP onset, VWF MM ratio decreased significantly and was low in all patients despite <10% ADAMTS13. The VWF MM ratio does not depend exclusively on ADAMTS13 activity. The disappearance of high molecular weight VWF MMs resulting in low VWF MM ratio at iTTP onset may be explained by consumption of larger VWF MMs in the microcirculation. The very high VWF MM ratio preceding acute iTTP recurrence suggests that VWF processing is hampered more than in patients remaining in remission.

Introduction

Immune-mediated thrombotic thrombocytopenic purpura (iTTP) is caused by a severe deficiency of ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 motifs 13) because of autoantibodies that inhibit or clear the protease.[1-3] In patients with severe ADAMTS13 deficiency, the

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The full-text version of this article contains a data supplement.

proteolytic processing of high molecular weight prothrombotic von Willebrand factor (VWF) multimers (MMs) into smaller, less adhesive molecules fails. Under high shear stress in arterioles and capillaries, VWF- and platelet-rich microthrombi occur[4-6] resulting in thrombocytopenia and microangiopathic hemolytic anemia with schistocytes. The microthrombi lead to organ ischemia, dysfunction, and clinical symptoms.[6-8] The acute iTTP episode is associated with high mortality and therefore requires rapid, appropriate therapy. Apart from therapeutic plasma exchange (TPE), plasma replacement and corticosteroids,[9,10] the anti-CD20 monoclonal antibody (mAb) rituximab[11-14] and since 2018, caplacizumab, a therapeutic nanobody binding to the A1 domain of VWF and preventing platelet binding and activation, became available.[15-17]

After an initial acute episode, up to 40% of survivors relapse over the ensuing years.[18,19] However, ADAMTS13 deficiency alone is not sufficient to trigger recurrence because some patients do not relapse despite consistently low ADAMTS13 levels. The complement system, inflammation, and cytokines seem to play an important, but currently not completely clarified, role in iTTP pathophysiology.[20-22] In addition, Roose et al demonstrated that ADAMTS13 is present in an open conformation during acute iTTP[23] and that open conformation in remission may herald recurrence.[24] In a mouse model using ADAMTS13 and VWF double-knockout mice (ADAMTS13$^{-/-}$/VWF$^{-/-}$), Chauhan et al[25] demonstrated that VWF deficiency abrogates the prothrombotic state of ADAMTS13 deficiency, suggesting that VWF is the indispensable mediator for platelet clumping.

VWF is secreted as an ultra-large MM, mainly from endothelial cells, and mediates platelet adhesion and aggregation at sites of vascular damage.[26,27] Cleavage by ADAMTS13 results in a typical MM pattern of higher and lower molecular weight MMs in plasma.[26,27] High molecular weight multimers (HMWM) (~5500-10 000 kDa) are more adhesive than lower molecular weight multimers (LMWM), and excessive cleavage reduces the ability of VWF to recruit platelets.[4,6,28] Unusually large VWF MMs (>10 000 kDa) are particularly adhesive and observed in the plasma of patients with TTP as a result of low ADAMTS13 activity. They are detectable only in remission and disappear during the acute episode, most likely because of the consumption in microthrombi.[29,30] For ~3 decades the analysis of ultra-large VWF MMs has been a part of the extended diagnostics in TTP,[29] but the method is laborious, requires a lot of expertise, and is therefore rarely offered for diagnostic purpose. The complicated procedure is not standardized and shows high gel-to-gel variability, even in the hands of the same investigator.[31] The Hydragel method is a well-standardized precast agarose gel system for VWF MM analysis.[32-36] Although the resolution of the gel is not sufficient for assessing the triplet structure of LMWM bands, the detection of absent HMWM is reliable and thus von Willebrand disease types 2A and 2B can be distinguished from type 1.[32,36,37] Ultra-large VWF MMs cannot be clearly separated from physiologic HMWM, but densitometric quantification of 3 predefined MM ranges (HMWM, intermediate molecular weight multimers [IMWM], and LMWM) are so reliable that reference ranges have been established.[27,32,35,37] Therefore, we investigated whether quantification of a newly defined fraction of HMWM compared with LMWM is an appropriate marker for ultra-large VWF MMs and may, in conjunction with other risk markers (ADAMTS13 activity and ADAMTS13 conformation index [CI]), be used as prognostic follow-up marker in iTTP.

Patients and methods

In the study period from July 2016 to August 2018, 91 patients with suspected or known iTTP were screened. One patient refused to participate. Of 90 initially included patients, 83 were confirmed to have iTTP (Figure 1). In 7 patients with suspected iTTP, a different diagnosis was made shortly after inclusion (Figure 1A). Of the 83 patients with iTTP, 8 were enrolled in the study during an acute episode including 4 with a first iTTP bout (Figure 1B). Seventy-five patients were enrolled during clinical remission. After inclusion in the study, 14 acute relapses occurred in 10 different patients during follow-up. Therefore, a total of 22 acute episodes were recorded in 16 patients over the study period (Figure 1B).

According to the outcome criteria proposed by an International Working Group on TTP,[38] acute TTP relapses are defined as platelet decrease (<150 000 cells/μL), LDH increase (>400 U/L), with or without clinical symptoms occurring in remission. This latter is defined as normalized platelets (>150 000cells/μL) and LDH <1.5× upper normal limit after discontinuing TPE and is sustained for >30 days.[38] Our cohort study ended before the introduction of caplacizumab into clinical practice obviating the need to apply the revised outcome criteria.[39]

Optimally, patients in remission were followed up as outpatients from every 3 to 4 months (52%), sometimes more often if a pending relapse was suspected, and others (34%) were seen only once a year. Patients in remission received a clinical examination, and blood was drawn for laboratory analysis and biobanking (supplemental Methods). Patients with acute iTTP were hospitalized and received a comprehensive laboratory analysis and biobanking before the first TPE. Laboratory routine testing and biobanking were performed daily before the TPE session for 10 days and twice a week thereafter until hospital discharge.

The prospective study was approved by German law (Landeskrankenhausgesetz §36 and §37) in accordance with the Declaration of Helsinki and by the ethics committee of the "Landesärztekammer Rheinland-Pfalz" (837.506.15 [10274]).

Controls

Forty pseudonymized patient controls and 25 controls were analyzed for deriving a reference VWF MM ratio (supplemental methods).

Assays (see also supplemental Methods)

VWF antigen and VWF activity. VWF antigen (VWF:Ag) as well as activity (VWF:Ab)[40] were measured at the University Medical Center Mainz using an ACL Top 750 coagulation analyzer (Instrumentation Laboratory/Werfen Diagnostics). In these immunoassays, VWF:Ag agglutinates with the polyclonal antibody–coated latex particles. Agglutination is proportional to the amount of VWF:Ag and is determined photometrically. VWF:Ab was also determined by an immunoturbidimetric assay, but the latex particles were coated with a mAb against the glycoprotein Ib binding site of VWF (HaemosIL VWF activity).[40]

ADAMTS13 activity and antigen. ADAMTS13 activity (ADAMTS13:act) was examined in Mainz using the fluorescence resonance energy transfer (FRET) method[41] (Peptide Institute, INC FRETS-VWF73 substrate) modified as described.[42,43]

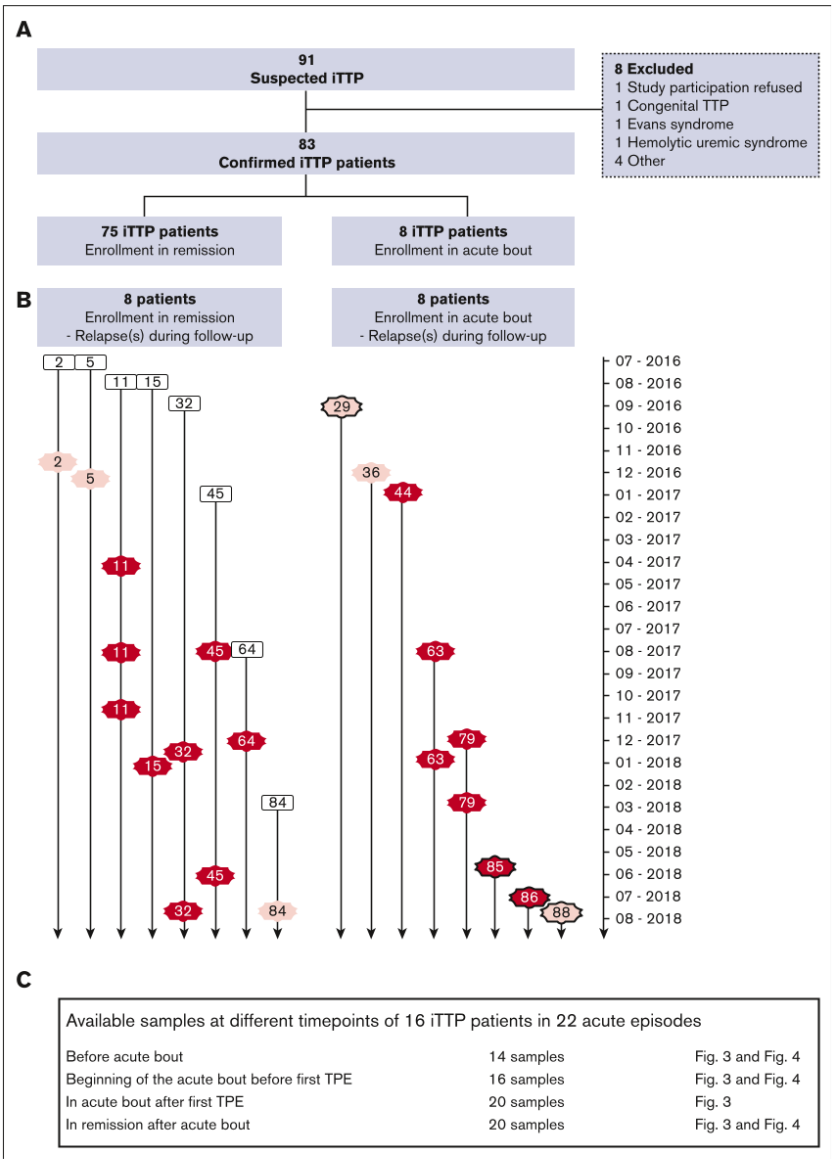

Figure 1. Recruitment of patients with iTTP cohort highlighting all acute iTTP episodes occurring during the prospective study. (A) Overview of patients enrolled in remission and during an acute iTTP episode. (B) All patients suffering 1 or several acute bouts are represented over the whole study period (time axis from July 2016 to August 2018 indicated on the right side). The left side of the panel shows the 8 patients enrolled in remission who suffered from relapse(s) during follow-up (black outlined boxes with patient numbers #2, 5, 11, 15, 32, 45, 64, 84). The red and pink stars show the acute relapse(s). The right side of the panel shows the 8 patients enrolled in acute episodes, indicated by red and pink stars (patient numbers #29, 36, 44, 63, 79, 85, 86, 88). The black outline of the stars indicates initial diagnoses (patients #29, 85, 86, 88). Pink stars indicate the acute episodes of patients who received treatment prior to biobanking. No blood samples were available from them at begin of the acute bout before first TPE (refer to panel C below). Red stars mark acute bouts with complete data and biobanking. (C) Summary of all available samples of 16 patients with 22 acute iTTP episodes at various time points (days-weeks before, acute iTTP onset before first TPE, during acute iTTP after first TPE, in remission after acute iTTP).

ADAMTS13 activity was expressed as a percentage of pooled normal plasma. The normal range in healthy donors is >50% with a detection limit of 1%.

ADAMTS13 antigen (ADAMTS13:Ag), experiment conducted in KU Leuven Campus Kortrijk, Belgium, was measured by an in-house developed ADAMTS13 antigen enzyme-linked immunosorbent assay (ELISA) as previously described.[23,44] In brief, plasma samples and a dilution series of normal human plasma (NHP) were added to a 96-well plate coated with mouse mAb 3H9.[45] Captured ADAMTS13 was colorimetrically detected using a mixture of biotinylated murine mAbs, 17G2 and 19H4[46] and horseradish peroxidase (HRP)–labeled streptavidin (Roche Diagnostics). The ADAMTS13 antigen levels were interpolated from the NHP reference.

ADAMTS13 conformation ELISA. ADAMTS13 conformation in plasma samples, conducted in Kortrijk, was determined using an in-house developed conformation ELISA as previously described.[23,24] In brief, 96-well plates were coated with the murine mAb 1C4 (5 µg/mL) which recognized a cryptic epitope in the spacer domain. After blocking, plasma samples were added in a 1 over 4 starting dilution and further diluted in a 1 over 2 dilution series. A dilution series of NHP, preincubated with the opening murine mAb 17G2,[46,47] was used for calibration. Detection of bound ADAMTS13 using biotinylated 3H9[45] followed by HRP-labeled streptavidin and colorimetric reaction allowed assaying open ADAMTS13 and calculating CI. A minimum of ≥0.02 µg/mL ADAMTS13:Ag is needed for CI assay. A CI of >0.5 indicates an open ADAMTS13 conformation whereas a CI of ≤0.5 indicates a closed ADAMTS13 conformation.

ADAMTS13:act, ADAMTS13:Ag, and CI of some iTTP samples had been analyzed for a previous study and were already published in this context.[24]

VWF MM ratio. VWF MM analysis was performed in Mainz using the Hydragel 11 von Willebrand Multimers kit (Sebia GmbH, Fulda, Germany) on the Sebia Hydrasis 2 system (Figure 2A). Plasma samples were thawed at room temperature, diluted with sample diluent (Sebia), incubated at 45°C for 20 minutes, adjusted to room temperature for a maximum of 10 minutes and loaded onto a precast agarose gel (sample volume 5 µL). To avoid saturated, not quantifiable MM bands, the samples were not only roughly diluted according to VWF:Ag concentration ranges (as suggested by the manufacturer) but were also adjusted exactly to 20% VWF:Ag. On each gel, 1 healthy control and 10 patient samples were separated. Electrophoresis, in-gel immunofixation and VWF staining, scanning of the gel, densitometry (Software Phoresis version 6.3, Sebia), and quantification by integrating the areas under the curves were part of a semiautomated workflow optimized by the manufacturer. Preliminarily, the electrophoretic pattern of VWF MMs was divided into following 3 zones according to the molecular weight: LMWM, 3 bands; IMWM, 4 bands; and HMWM. Because the manufacturer's classification was not optimized for the use in TTP, we complemented the MM classification by splitting HMWMs in 2 separate categories, the anodal 2 bands (HMWM1) and the residual-HMWM bands (HMWM2) (Figure 2A).

Statistical analysis

Clinical symptoms and medical history of each patient were recorded in Open Clinica. Laboratory parameters and biobanking were recorded in Nexus/Swisslab (version 2.22.5.00). Statistical analysis was performed using GraphPad Prism 9. The descriptive statistics included frequency, mean, standard deviation, median, interquartile range, and minimum and maximum. The differences between 2 groups were tested using Student *t* test for normally distributed data and the nonparametric Mann-Whitney *U* test for nonnormally distributed data. Any *P* values <.05 were considered to be statistically significant.

Results

Characteristics of the enrolled patients with iTTP

Eighty-three patients with confirmed iTTP were included (Table 1). The majority of 61 (73%) were female. Patients were followed for a median of 468 days and were aged 49 years (median) at study inclusion. The patients were first diagnosed with iTTP at a median age of 38 years (range 12-85 years). The median number of previous acute episodes before inclusion in the study was 2. Four patients were enrolled during their initial iTTP episode, 2 of them directly admitted to the University Medical Center Mainz and 2 transferred from external hospitals. Five (7%) patients had already had ≥10 acute prior episodes.

Data on the acute iTTP episodes

Of the 22 acute episodes recorded in 16 patients during the study period, complete clinical and laboratory data and biobanking of plasma samples before TPE were available for 16 episodes in 10 patients (Figure 1B-C). All 16 well-documented acute episodes were characterized by severely decreased ADAMTS13 activity, thrombocytopenia, increased LDH, and signs of microangiopathic hemolysis (Table 2). The severity of the acute bouts assessed by our clinical score[48] was mild in 5, moderate in 8, and severe in 2 instances, and 1 patient with 3 previous acute episodes showed only laboratory signs of acute TTP (Table 2; supplemental Table 1). For all 16 acute iTTP episodes, from 4 to 29 TPEs were performed over 8 to 36 days, most patients received prednisolone and 5 rituximab (supplemental Table 1). All patients survived. Comorbidities besides iTTP are listed in supplemental Table 2.

Follow-up of all patients with iTTP and development of the VWF MM ratio

We monitored all 83 patients with iTTP (Figure 1A) for clinical signs and symptoms, blood values, ADAMTS13 parameters, LDH and VWF MM pattern over the study period up to 2 years. An example for 1 closely followed patient is shown in supplemental Figure 1. VWF MM pattern turned out as potentially interesting parameter (see below).

Set up and evaluation of a new VWF MM ratio. To define an informative VWF MM fraction including predominantly ultra-large MMs, the HMWM fraction was split in 2 zones, the anodal 2 bands (HMWM1), which could be clearly differentiated in all lanes, and the remaining HMWM2 (Figure 2A). After analyzing the 65 control samples and the first 77 samples of patients with TTP and quantifying the predefined fractions, the preliminary evaluation revealed that, among all fractions, HMWM2 and LMWM differed most pronouncedly between patients with iTTP and controls, expressed by increased HMWM2 and decreased LMWM in TTP. IMWM were slightly decreased in patients with iTTP and no

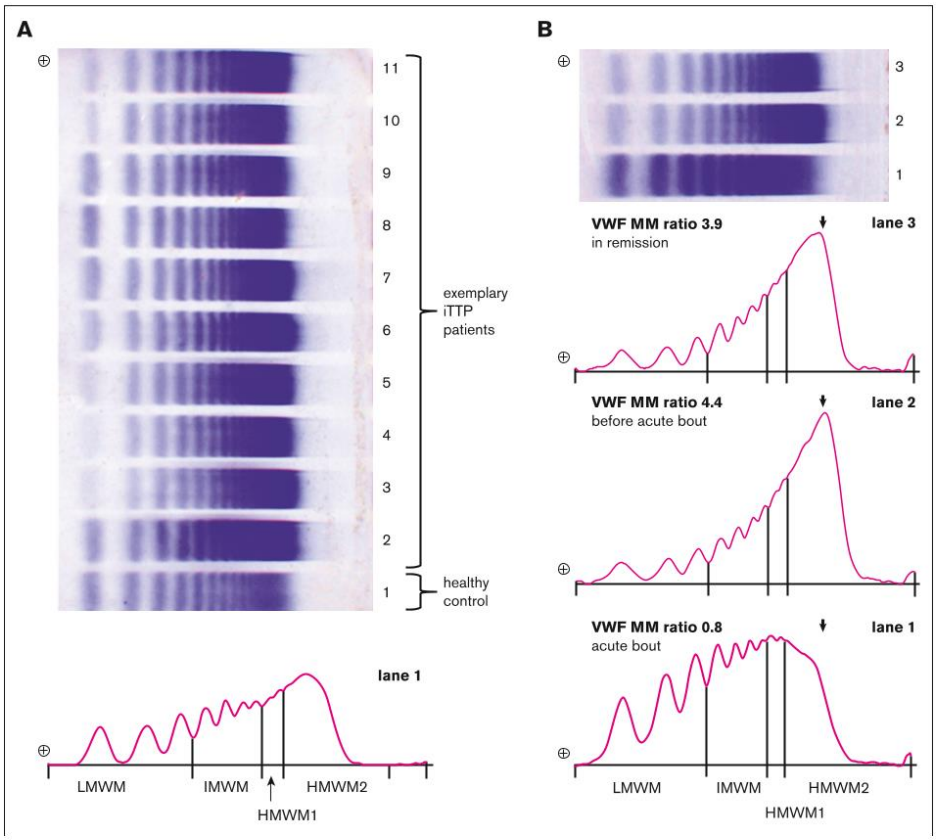

Figure 2. VWF MM ratio evaluation. (A) Exemplary VWF MM gel with plasma samples from patients with iTTP (lanes 2-11) and from a healthy control (lane 1). The densitometry trace below the stained gel represents lane 1 with its electrophoretic pattern divided in 4 zones according to the molecular weight: LMWM; IMWM; HMWM1; and HMWM2. The VWF MM ratio is defined as HMWM2/ LMWM. (B) Electrophoretic patterns and densitometry traces of 1 patient with iTTP in the course of the disease. Lane 3: in remission; lane 2: before acute bout; lane 1: in the acute bout before first TPE.

difference was observed for HMWM1. Therefore, the ratio of HMWM2 to LMWM (VWF MM ratio) was considered as the most discriminative parameter (Figure 2B).

Set up and coefficient of variation (CV) of VWF MM ratio. On 38 gels, each with 11 slots, 1 aliquot of a total of 4 individual healthy control samples was analyzed in parallel with sample of 10 patients, respectively. For healthy control #1, a VWF MM ratio of 1.7 was found with a CV of 15% (n = 26 gels). This was confirmed with healthy controls #2 to 4 with VWF MM ratio 1.9, CV 19% (n = 6), VWF MM ratio 1.3, CV 15% (n = 3), and VWF MM ratio 1.3, CV 14% (n = 3), respectively. Furthermore, 20 samples of patients with iTTP were analyzed and repeated at another day: The relative deviation of the recorded duplicates (calculated as $|d| \times 100/m$; d: absolute value of the duplicates' difference, m: mean of the duplicates) was found to be 9.12% ± 7.03% (mean ± standard deviation).

Set up and sample stability to freeze-thaw cycles. One gel was loaded with 5 technical replicate samples of each, 1 healthy control, and 1 patient with TTP. Replicates had been subjected to 1 to 5 freeze-thaw cycles. A tendency neither toward higher nor toward lower VWF MM ratios was observed (healthy control: VWF MM ratio with increasing freeze-thaw cycles: 1.8, 2.4, 1.9, 1.8, 1.7, CV 14%; patient with TTP: VWF MM ratio with increasing freeze-thaw cycles: 4.9, 4.7, 5.0, 4.9, 4.1, CV 8%).

Set up and patient controls and healthy controls. Forty pseudonymized patient controls and 25 anonymized healthy controls were analyzed. To ensure optimal predilution of the samples for the VWF MM gels, VWF:Ag was determined in all controls (patient controls: mean ± standard deviation 156% ± 55%; minimum 77%, maximum 275%; healthy controls: mean ± standard deviation 108% ± 32%; minimum 59%, maximum 170%). The

Table 1. Characteristics of the cohort of patient with iTTP

Characteristics	n	%
Total number of patients with iTTP	83*	
Sex		
Female	61	73
Male	22	27
Ethnicity		
Caucasian	83	100
Observation time, d		
Median (minimum, 25th percentile, 75th percentile, maximum)	468 (1, 56, 680, 782)	
Age at time of enrollment, y		
Median (minimum, 25th percentile, 75th percentile, maximum)	49 (20, 39, 57, 86)	
Age at time of diagnosis of first acute iTTP episode, years†		
Median (minimum, 25th percentile, 75th percentile, maximum)	38 (12, 27, 49, 85)	
Number of acute TTP episodes during study	22 in 16 patients	
Patients enrolled during acute iTTP	8	
Initial diagnosis during study	4	
Relapse of iTTP	4	
Relapses during follow-up after enrollment	14	
Number of patients with iTTP in continued remission during study	67	
Number of all acute episodes per patient before enrollment into the study‡		
Median (minimum, 25th percentile, 75th percentile, maximum)	2 (0, 1, 3, 23)	
Risk factors for iTTP		
Smoking	17	20
Overweight (BMI, 27-29.9)§	12	16
Obesity (BMI >30)§	26	34
Arterial hypertension	28	31
Type 1 diabetes mellitus	1	1
Type 2 diabetes mellitus	11	13

BMI, body mass index.
*Ninety-one patients were screened (refer to supplemental Figure 1).
†Known for 80 patients with iTTP.
‡Known for 75 patients with iTTP.
§Known for 76 patients with iTTP.

VWF MM ratio values were normally distributed for both collectives. In patient controls a mean ratio of 2.3 ± 0.6 (SD) was detected, in healthy controls 2.3 ± 0.7. Because results were very similar in both control groups, the reference range for the VWF MM ratio was calculated from a total of 65 controls: 1.1 to 3.5 (2.5th-97.5th percentile).

Course of VWF MM ratio in patients with iTTP with and without acute episodes

The VWF MM ratio typically increased before the acute iTTP bouts and dropped at onset of the acute episode (Figure 3). The values of VWF MM ratio before acute episodes were significantly higher than in controls ($P < .0001$), but also compared to patient with iTTP who did not experience an acute bout (Figure 4). At the onset of an acute episode, the VWF MM ratio dropped significantly ($P = .0003$) to values often below the lower limit (<1.1). In contrast, both patient with iTTP in continuing remission and healthy controls showed a constant VWF MM ratio (supplemental Figure 2). Toward remission (median 58 days after last TPE), the VWF MM ratio values normalized and exhibited no difference to the control group (Figures 3 and 4).

Comparison of VWF MM ratio, VWF antigen, ADAMTS13 activity, and ADAMTS13 CI in patient with iTTP with and without an acute episode during the study period

To detect potential differences between patients with and without acute episodes, the patients were divided into following 2 groups: those with (n = 15) and without (n = 67) acute episodes during the study period. The 67 patients remaining in remission for the complete study period were further divided into those with ADAMTS13:act ≥10% (n = 54) and <10% (n = 13). In addition, 65 healthy and patient controls were also analyzed for comparison (Figure 4).

VWF MM ratio was assessed in remission before an acute episode in 14 instances (10 different patients), at onset of acute iTTP bout in 16 instances (10 patients), and during remission after an acute iTTP in 20 instances (14 patients) (Figures 1C and 4). Patients in continuing remission with ADAMTS13:act ≥10% had a significantly lower VWF MM ratio than those with ADAMTS13:act <10% ($P = .0023$). Nevertheless, patients with ADAMTS13:act below ($P < .0001$) as well as above 10% ($P = .0012$) showed significantly higher VWF MM ratio than controls. Fourteen samples obtained 39 days (median; min. 2, interquartile range 13-50, max. 196 days) before acute iTTP (ADAMTS13 <10% in 9, 10%-26% in 5) showed significantly higher VWF MM ratios than those of 13 patients in continuing remission with ADAMTS13 <10% ($P = .0020$), as well as those of the 54 patients in remission with ≥10% ($P < .0001$). Sixteen samples of patients with acute iTTP displayed significantly lower VWF MM ratios than the 13 patients with iTTP in remission with an ADAMTS13 <10% ($P = .0033$). The VWF MM ratio in patients with iTTP in remission after an acute bout was comparable to that of controls and lower than in patients with iTTP in continuing remission.

Bernardo et al[20] demonstrated that inflammatory cytokines stimulate the release of HMW VWF MM from human umbilical vein endothelial cells and that interleukin 6 (IL-6) inhibits their cleavage under flow conditions. Therefore, we examined C-reactive protein (CRP) and IL-6. CRP levels in patients before an acute bout were equal or lower than in patients in continuing remission (supplemental Figure 3). IL-6 levels were not detectable before an acute episode and were significantly higher in patients with iTTP in remission ($P = .0124$ and $P = .0162$), although still within the normal range (<7 pg/mL) (supplemental Figure 3). At the beginning of an acute bout, CRP and IL-6 were significantly higher than at all other time points and compared with that of patients with iTTP patients in remission. Thus, no correlation between these inflammatory parameters (CRP and IL-6) and high VWF MM ratio was established. The VWF antigen levels in patients were higher before, at onset, and after the acute episode than patients in remission and controls (Figure 4C). As previously reported,[24] in patients in continuing remission over up to 2 years, all 13 patients with an

Table 2. Laboratory parameters of 16 well-documented acute iTTP episodes in 10 patients

			Laboratory parameters at onset of the acute iTTP episode														
Pat.ID	Sex	Severity of the acute episode (clinical score)*	Platelets [cells/mL] 150-360	Hemoglobin [g/dL] m 13.5-17.5 f 12.0-16.0	Schistocytes [%] <5%	Haptoglobin [g/L] m 0.14-2.68 f 0.11-2.73	LDH [U/L] <245	VWF antigen [%] 42-176	VWF activity [%] 40-170	VWF MM ratio 1.1-3.5	ADAMTS13 activity [%] >60	ADAMTS13 antigen [µg/mL] >0.5	ADAMSTS13 CI <0.5	ADAMTS13 inhibitor [BU/mL] <0.5	IL-6 [pg/mL] <7	TNI [pg/mL] <24
11	F	Moderate (2)	97	11.1	10	0.09	307	242	336	3.1	1.4	0.24	7.57	0.3	<2	2.6
11	F	Moderate (2)	54	7.2	17	<0.08	609	216	373	3.1	2.6	0.25	0.48	0.8	3	252.6
11	F	Moderate (2)	65	9	5	0.82	444	228	>390	2.6	5.7	0.22	0.49	0	3	85.9
15	F	Laboratory abnormalities only (0)	17	11.7	3	<0.08	506	214	199	2.6	<1.0	0.07	8.49	1.0	3.53	<1.95
32	M	Moderate (2)	20	10.8	15	<0.08	527	226	215	2.2	<1.0	0.60	1.06	0.6	2	1.8
32	M	Moderate (2)	60	12.2	4	<0.08	505	227	283	3.2	<1.0	0.09	13.45	1.3	<2	9.4
44	M	Moderate (2)	88	9.9	14	<0.08	612	216	159	3.1	<1.0	0.20	3.9	2.6	3	133.6
45	F	Mild (1)	30	9.6	17	<0.08	413	106	70	0.7	1.6	0.08	0.33	0.9	<2	15.7
45	F	Moderate (2)	11	10.3	20	<0.08	697	162	130	0.9	<1.0	0.08	0.39	2.6	3	4.4
63	F	Mild (1)	29	10.4	17	<0.08	488	68	43	0.4	<1.0	0.06	9.56	0.7	3	1.8
63	F	Mild (1)	39	11.0	4	<0.08	297	78	69	2.8	4.8	0.17	5.13	1.4	<2	1.6
64	F	Mild (1)	49	10.2	11	<0.08	560	243	235	1.8	2.2	0.11	4.32	0.6	44	53.9
79	F	Moderate (2)	17	7.4	38	not done	749	177	149	0.5	<1.0	0.37	1.51	1.8	9	180.6
79	F	Mild (1)	11	7.8	31	<0.08	634	99	74	1.4	1.3	0.08	1.36	1.7	3	13.9
85	F	Severe (3)	6	6.9	23	<0.08	1678	261	not done	1.1	<1.0	0.05	4.03	1.2	14	2236.3
86	M	Severe (3)	8	10.1	15	<0.08	1165	230	122	0.6	<1.0	0.03	3.95	2.6	24	13.2

BU, Bethesda Units; F, female; LDH, lactate dehydrogenase; M, male; Pat.ID, patient identification; TNI, troponin I.
*Clinical severity score for acute iTTP episodes.[48]

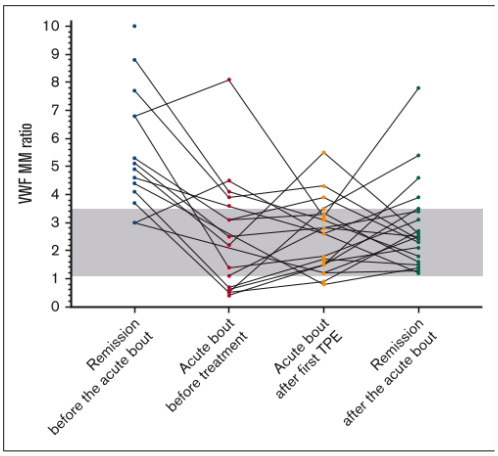

Figure 3. VWF MM ratio before, during and after acute iTTP episodes. VWF MM ratios in patients with iTTP from 2 days to 28 weeks (median 39 days) before an acute bout (n = 14), at the onset of the acute iTTP episode (n = 16), after first TPE (n = 20), and in remission after the acute episode (n = 20). Gray background is the normal range of the VWF MM ratio assessed in controls.

ADAMTS13 activity <10% showed an open ADAMTS13 conformation. In those in continuing remission with ADAMTS13 activity >10%, the conformation was open in 50%. It appeared that the higher the ADAMTS13 activity the more likely it is that ADAMTS13 has a closed conformation (Figure 4B). In 13 of 14 (93%) samples obtained before an acute episode, an open ADAMTS13 conformation was evident. At the beginning of the acute bout, 12 of 16 (75%) cases showed an open ADAMTS13 conformation whereas in remission, after the acute iTTP, only 50% of cases still had an open ADAMTS13 conformation (Figure 4B). Interestingly, the lower the VWF MM ratio and the higher ADAMTS13 activity, the more likely it is that the ADAMTS13 conformation is closed (Figure 4A-B).

Discussion

The goal of this prospective single center cohort study on patients with iTTP recruited and followed at the University Medical Center in Mainz between July 2016 and August 2018 was to identify markers associated with the disease course. Patients were enrolled during hospitalization for acute iTTP episodes or as outpatients followed in remission at ~3-month intervals. Clinical and laboratory data were documented, and biobanking of plasma samples was performed. Caplacizumab, nowadays recommended[14,49,50] and used at many specialized centers in parallel with TPE and corticosteroids during hospitalization for acute iTTP,[17,51-53] was not yet available during the study period. Rituximab, now recommended both upfront in acute iTTP episodes as well as preemptively in clinical remission when ADAMTS13 activity drops below 10% to 20%,[12,14,50,54,55] was not yet uniformly used in this cohort. Rituximab was given to 5 patients who were frequently relapsing during an acute iTTP episode and only to 1 patient in clinical remission with a severely deficient ADAMTS13 activity.

Persistent or recurrent deficiency of ADAMTS13 activity during follow-up of survivors of acute iTTP is an established risk factor for disease recurrence.[18,56] However, 13 of the 83 included patients in our cohort remained in clinical remission for up to 2 years despite severely deficient ADAMTS13 activity of <10%. Open ADAMTS13 conformation, that is a CI of >0.5, a specific hallmark of iTTP[23] was recently shown to be caused by autoantibodies binding to ADAMTS13.[24] Also, long-term follow-up of 1 patients with iTTP suggested that open ADAMTS13 conformation might precede ADAMTS13 activity decrease[24] and thus may be a potential early indicator of impending iTTP relapse. Interestingly, 13 of 14 patients, from 2 days to 28 weeks before an acute clinical relapse showed a CI >0.5, nevertheless all 13 patients with ADAMTS13 <10% and half of those with ADAMTS13 ≥10% remaining in clinical remission during the entire study period had an open ADAMTS13 conformation as well. However, our study did not focus on the comparative predictive value of ADAMTS13 activity, CI, and the VWF MM ratio because of limited number of patients and short follow-up. The ADAMTS13 CI is currently being tested as predictive marker for biological or clinical relapse in a multicenter cohort of patients in remission and normal ADAMTS13 activity (de Waele, Sakai et al manuscript in preparation).

Based on the fact that the hyperadhesive, ultra-large VWF directly mediates microvascular platelet adhesion and aggregation and clinical and laboratory signs of acute TTP in animal models,[25,57] in this study we focused on the plasmatic VWF MMs of our patients with iTTP. Although standardization of VWF MM analysis seems to be still challenging,[31] the commercially available Hydragel 11 VWF MM kit represents a reproducible assay to analyze the VWF MM distribution. Using constant amounts of VWF for electrophoresis samples, preliminary testing of a series of iTTP and control samples allowed to quantitate high-, intermediate- and low-molecular weight VWF MMs, with the high molecular weight VWF MMs split in a HMWM1 and a HMWM2 fraction. The densitometrically assessed ratio of HMWM2 to LMWM was reproducibly quantified and differed between normal and iTTP plasma samples and between different disease states in patients with iTTP.

First, the VWF MM ratio differentiated patients with iTTP in remission with <10% ADAMTS13 activity from those with ≥10%. Moreover, at the onset of acute iTTP before the first TPE, the VWF MM ratio decreased, often to subnormal values which may be explained by consumption of predominantly large and ultra-large VWF MMs during the acute microvascular thrombotic process.[29,30] The most interesting finding was that plasma samples obtained a few days to several weeks before acute disease relapse showed significantly higher VWF MM ratios than to those from patients remaining in continuous remission. The reason for this unexpected finding of very high VWF MM ratios despite measurable ADAMTS13 activity (10%-26% of normal) in 5 of 14 cases, remains elusive. IL-6 has been reported to inhibit VWF cleavage by ADAMTS13 under flow conditions.[20] IL-6 and CRP levels were not elevated in samples before acute iTTP relapse and could not explain this phenomenon. However, our data suggest that VWF proteolytic processing may be impaired in distinct patients with iTTP even in the presence of measurable ADAMTS13 activity and that those patients may be at high risk of experiencing a clinical iTTP relapse.

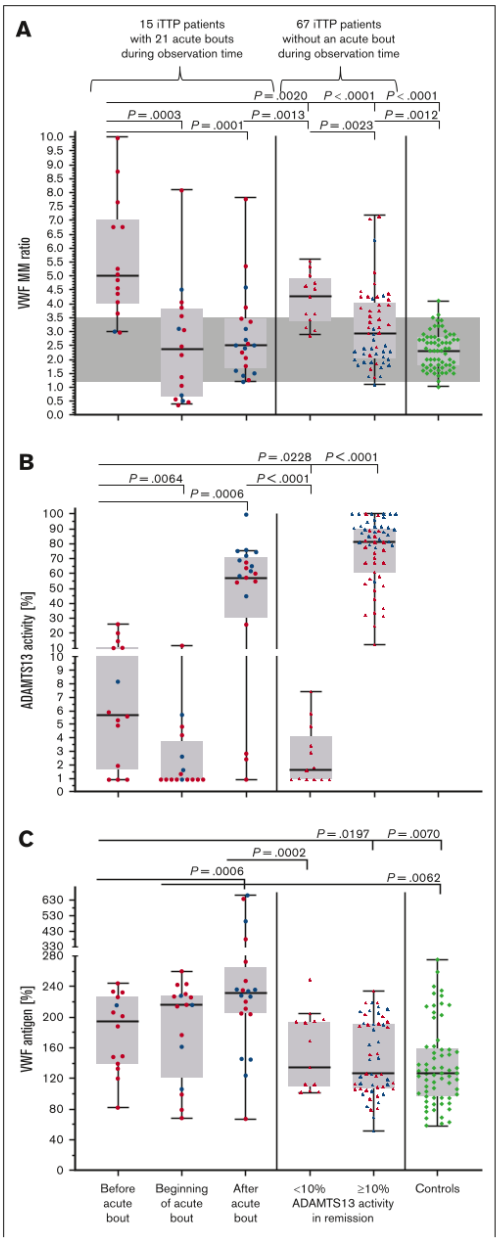

Figure 4. VWF MM ratio, VWF antigen, ADAMTS13 activity, and ADAMTS13 CI in patients with iTTP. The VWF MM ratio (A), ADAMTS13 activity (B) and VWF antigen (C) in 82 patients with iTTP with and without acute episodes during the study period, as well as in 65 controls. On the left side of panels A, B, and C, the VWF MM ratio, ADAMTS13:act, and VWF:Ag values are shown before (n = 14), at the beginning (n = 16) as well as in remission after the acute episode (n = 20). On the middle panel, patients with iTTP who were constantly in remission are grouped according to ADAMTS13 values <10% (n = 13) and ≥10% (n = 54). An open ADAMTS13 conformation is marked with red symbols, and a closed one is marked with blue symbols in panels A-C. Controls are shown on the right side of panels A and C. Gray background is the normal range of the VWF MM ratio assessed in controls.

Our study is relatively small with limited numbers of patients developing a clinical relapse, and the follow-up time with maximally 26 months was rather short. However, the hypothesis is raised that VWF MM distribution, in addition to decreased ADAMTS13 activity and open ADAMTS13 conformation, may be an important marker heralding acute disease manifestation in patients with iTTP. A large, prospective, probably multicenter study with regular follow-up of patients with iTTP over several years should be undertaken to verify the prognostic role of the VWF MM ratio and to hopefully clarify its pathophysiology.

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Authorship

Contribution: T.F. developed study concept, analyzed and compiled data, and wrote and approved the manuscript; H.R. developed study concept and design, was responsible for assay evaluation, and wrote and approved the manuscript; L.d.W. and C.D. performed data acquisition and analysis (ADAMTS13 conformation and antigen), and revised and approved the manuscript; C.v.A. developed study concept and carried out approval and enrollment of the patients; N.M.-C. performed graphical representation and analysis and revised and approved the manuscript; F.H. performed data acquisition (ADAMTS13 activity) and revised and approved the manuscript; A.D. performed data acquisition (VWF multimer gels) and approved the study; D.M. performed blood sampling for biobanking, clinical data acquisition, and revised and approved the manuscript; X.M. performed clinical data acquisition and approved the study; M.S. performed clinical data acquisition and revised and approved the manuscript; K.J.L. provided statistical advice and revised and approved the manuscript; K.J. performed data acquisition and analysis, and revised and approved the manuscript; K.V. developed study concept, performed data analysis and acquisition, and revised and approved the manuscript; and B.L. developed study concept and design, performed data analysis, and wrote and approved the manuscript.

Conflict-of-interest disclosure: B.L. is chairman of the data safety monitoring committees for the Baxalta 281102 and TAK-755-3002 studies (both investigating recombinant ADAMTS13 in hereditary TTP) and for the Takeda SHP655-201 study (recombinant ADAMTS13 in immune-mediated TTP), all 3 now run by Takeda. B.L. was a member of the advisory board of Ablynx, now part of Sanofi, for the development of caplacizumab; is chairman of the data monitoring committee of the Mayari study, run by Sanofi, on iTTP patients; and received congress travel support and/or lecture fees from Baxter, Ablynx, Alexion, Siemens, Bayer, Roche, and Sanofi. The remaining authors declare no competing financial interests.

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