Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

Methylene blue-treated plasma, versus quarantine fresh frozen plasma, for acute thrombotic thrombocytopenic purpura treatment: Comparison between centres and critical review on longitudinal data

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ARTICLE INFO

ABSTRACT

Introduction: Therapeutic plasma exchange (TPE) is the first-line treatment for acute thrombotic thrombocytopenic purpura (TTP). Methylene blue-plasma (MBP) has been used for over 20 years, but its efficacy in this setting remains controversial.

Patients and methods: this is a comparative analysis of the experience of two Centres, with different plasma products, to evaluate their efficacy in TTP. One centre used quarantine plasma (QP), and MBP the other. We performed a retrospective longitudinal study, analysing the clinical files of TTP patients of a 13-year data evaluation period. Duration of treatment and transfusion parameters, medical record, laboratory testing, concomitant medication, and survival rate, were assessed for every episode.

Results: During the study period, 12 (55.5 %) and 10 (45.5 %) new cases were treated with QP and MBP, respectively. There were no significant differences between the mean numbers of TPE processes, days elapsed from diagnosis to TPE, and plasma volume transfused. The QP TPE episodes of treatment were significantly associated with an increased time to recovery compared with MBP episodes of treatment ($p = 0.004$).

Conclusion: MBP was as effective as QP in the treatment of TTP patients. Since recovery was more favourable when MBP was used, we consider MBP remains a suitable alternative to treat TTP patients.

1. Introduction

Thrombotic thrombocytopenic purpura (TTP) is a rare hematologic disease, initially described by Moschcowitz in 1924 [1]. Its incidence is very low (less than 5 cases/million/year) [2] and is a serious, life-threatening disorder characterised by severe thrombocytopenia (typically $< 30 \times 10^9$ platelets/L), negative direct antiglobulin test, microangiopathic haemolytic anaemia, multivisceral ischemic symptoms (usually neurologic changes, renal impairment, and fever), with no other apparent causes, being twice as frequent in women than in men [3]. It happens mostly during adulthood, with a tendency to recurrent relapses. Often, no associated clinical condition is found, but in half of the cases, TTP is diagnosed after bacterial or viral infections, autoimmune diseases or in association with drugs.

TTP is a consequence of a severe functional deficiency (activity <

10 %) of von Willebrand factor (vWF) disintegrin and metalloprotease with thrombospondin type 1 motif, member 13, (ADAMTS-13), either congenital or acquired associated with the presence of inhibitory antibodies [4]. As a result, the ultra-large vWF multimers are not cleaved; they will accumulate in the bloodstream promoting platelet (PLT) aggregation and intravascular thrombosis. Other pathophysiological conditions will be an additional factor to trigger the clinical syndrome.

TTP had a high mortality rate of about 90 % until the systematic implementation of therapeutic plasma exchange (TPE) in 1991 [5]. Although mortality has been drastically reduced, TTP has still a fatal outcome rate of 10 %–20 % [6]. TPE is an effective and safe treatment, considered the first-line therapy for acute TTP [7]. TPE removes the antibodies that inhibit ADAMTS-13 and supplies fresh ADAMTS-13. Concomitant steroids or Rituximab can be given depending on the clinical context.

Table 1
Plasma products in use for TTP treatment.

Type of plasma	Procedure	Safety Measure	Brand, Supplier	Reference
Fresh-frozen	Quarantine	Time. The donor must donate a second time longer than the window period and viral serology must remain negative	In house, Transfusion Centres	[8]
Photo-inactivated	Chemical Photosensitiser	Methylene Blue + visible light	THERAFLEX MB-Plasma, Macopharma	[9]
Pooled plasma	Organic Photosensitiser	Amotosalen + Ultraviolet A light	Intercept®, Cerus	[11]
	Industrial Solvent-Detergent	Plasma units are pooled and treated with lipid - degrading chemicals.	Octaplas®, Octapharma	[10]

Apart from quarantine fresh frozen plasma (QP) [8], several pathogen-reduced plasma products are available [9] (Table 1). Some suppliers use solvent/detergent-treated pooled plasma (OctaplasLG, Octapharma) [10]; others use a photosensitizer and light such as amotosalen and ultraviolet light (Intercept plasma, Cerus) [11], 2006) or methylene blue (MB) and visible light (THERAFLEX MB-Plasma, Macopharma). All are currently used and have demonstrated therapeutic efficacy and safety in TTP patients. Although the level of ADAMTS-13, after MB/light treatment, is normal [12] and similar to other pathogen-reduced plasmas or QP [13–15], the use of MB remains controversial, as some authors suggest it is less effective in terms of the volume of plasma required, than QP, in TTP patients [16–18], arguing that more TPE sessions and a larger amount of MB-Plasma, are required to achieve TTP remission.

MB/light is a photodynamic pathogen inactivation procedure for single units of fresh frozen plasma (FFP) [19,20]. THERAFLEX MB-Plasma system uses a photosensitive agent (MB) and visible light at 630 nm, to intercalate into the RNA/DNA backbone of the pathogen's genome. Many of the biological effects of MB are closely associated with its unique physicochemical properties, including its redox characteristics, ionic charges, and light spectrum characteristics. As a photosensitiser, MB can have two different mechanisms of action. Excitation by light can induce singlet and triplet stages of the molecule and transfer the energy through either electrons (Type I mechanism) or energy (Type II mechanism). The Type I mechanism can lead to the formation of hydroxyl radicals and lipid hydroperoxides. The Type II mechanism proceeds through singlet oxygen resulting mainly in breakages of nucleic acids, mostly at the guanosine site, leading to oxidation and destruction of the viral nucleic acid strand breakages preventing viral replication [20]. Especially, lipid peroxidation induced by MB photosensitization has detrimental effects on membrane integrity, leading to a loss of fluidity and alterations on the functions of several ion channels, receptors, and transporters.

The THERAFLEX MB-Plasma system has been proven highly effective in inactivating lipid-enveloped viruses, such as human immunodeficiency virus (HIV), hepatitis B and C viruses, and non-enveloped viruses such as Parvovirus B19, and arboviruses like West Nile virus [21], Dengue, Zika, Chikungunya [22,23]. Emerging viruses, such as the Middle East respiratory syndrome coronavirus and Ebola [24], and more recently, severe acute respiratory syndrome coronavirus (SARS-CoV), Crimean–Congo haemorrhagic fever virus and Nipah virus, have been effectively inactivated [25]. There's ample clinical experience using this product [26].

To shed some more light on the therapeutic impact of MB/light on safety and efficacy of plasma and being aware of the difficulties in developing a prospective study, two neighbouring regions of northern Spain, Asturias and Cantabria, fulfilled several favourable conditions to develop a longitudinal retrospective study reducing, as far as possible, the bias of selecting non-homogeneous populations. Both communities share common characteristics, such as proximity, geography, climate, resources, and history. The main difference, for our purpose, is that the first one uses MB-Plasma, as their standard to treat TTP patients, and the second one uses QP.

2. Material and methods

2.1. Inclusion criteria

A retrospective analysis began with the real-world data from the clinical health records of all the patients diagnosed with acute idiopathic TTP and treated with TPE, at two University Hospitals: Central de Asturias, Oviedo, Spain, and Marqués de Valdecilla, Santander, Spain, between January 1st of the year 2005 and December 31 of the year 2017.

The inclusion criteria were patients with a clinical diagnosis of TTP, based on suggestive clinical symptoms and microangiopathic haemolytic anaemia and thrombocytopenia ($< 100 \times 10^9$ PLT/L), with negative direct antiglobulin test and increased serum lactate dehydrogenase (LDH) level. Patients with microangiopathy related to other processes (tumours, infections, hematopoietic transplantation), or with insufficient information, were excluded from the database.

Demographic parameters were collected from all patient clinical records. Diagnosis, TPE treatment initiation and completion dates, time from diagnosis to start of TPE (days), were recorded for every episode, as well as the process date, transfused plasma volume (mL), PLT count (x 10^9/L) and LDH level (IU/L). Clinical information, laboratory testing for the diagnosis, concomitant medication and survival rate, were also obtained. Direct Coombs test, haptoglobin level (mg/dL), ADAMTS-13 activity and inhibitory autoantibodies by the collagen-binding affinity method were gathered from the medical records when available. The comparative survival rate and the cause of death were analysed for safety outcomes evaluation. The total plasma volume transfused during the TPE treatment period was included in the comparison analysis.

Criteria to claim complete response to TPE treatment was the combination of a full clinical recovery, PLT count returned to normality ($> 150 \times 10^9$ PLT/L), and serum LDH level reverted within the normal range (< 410 IU/L), for at least two consecutive days.

In this assessment, the group labelled as "QP group" corresponds to all medical records from patients evaluated in Cantabria, who were treated with quarantine plasma. QP from FFP was produced at the Banco de Sangre y Tejidos de Cantabria, Liencres, Spain, according to the Council of Europe recommendations (Keiter, 2015). The "MBP group" includes all the documented patients in Asturias treated with THERAFLEX MB system - inactivated plasma. MB/light-based, THERAFLEX MB-Plasma pathogen reduction system (Macopharma, Mouvaux, France), was supplied by the Centro Comunitario de Sangre y Tejidos de Asturias, Oviedo, Spain, following the manufacturer specifications.

2.2. Plasma sources for therapeutic use. Therapeutic protocol

Plasma units are obtained from volunteer donors, either by apheresis or from whole blood.

Quarantine plasma is stored frozen until the donor returns a second time, at least four months after the initial donation. If the viral serologic markers are still negative, the transfusion software will lift the quarantine, and the plasma will be passed into available stock.

The THERAFLEX MB-Plasma system consists of Plasmaflex, a leukocyte depletion filter that eliminates intracellular viruses by removing

residual cells present in plasma, and a MB pill that dissolves in the filtered plasma product. After illumination, an optional Blueflex filter, attached to the bag system, removes about 90 % of the residual MB and its photoproducts [9].

Both Centres used the Cobe Spectra system (Terumo BCT, USA) for TPE, running the therapeutic automated plasmapheresis protocol. Each treatment session aimed to exchange one blood volume. The processed blood was anticoagulated with ACD-A (1/9 v/v) as it came into the disposable kit and anticoagulation was reversed in the outflow line with equimolar calcium and magnesium chlorides.

2.3. Statistical analysis

Continuous variables are represented by descriptive statistical parameters, such as the number of cases, arithmetic mean, median and standard deviation (SD). The qualitative variables are characterised by the number of observations of their categories (absolute frequencies) and their relative frequencies (as a percentage).

Before the comparative analysis between groups, the goodness of fit to a Gaussian distribution of each quantitative parameter has been verified, in each group and subgroup, using the Kolmogorov-Smirnov test.

The homogeneity between the groups treated with QP and MBP, or other subgroups, has been tested, for the baseline characteristics and the rest of the parameters evaluated, using the Student *t*-test or the Mann-Whitney test for independent samples, in the same direction (QP values *minus* MBP values), calculating the 95 % confidence interval (CI), the difference of means (D) and the standard error of the difference (SE), for quantitative parameters. The Pearson Chi-square test or the Fisher exact test were used for the qualitative parameters. All the comparative tests performed were considered statistically significant when the probability of error (*p*) was < 0.05.

The first relapse variable was taken into account to compare demographical information only. Every episode was considered independent and evaluated separately. The days of TPE treatment (time to recovery) between TPE groups was assessed by the Mantel-Cox test for the comparison of survival curves.

The package of statistical programs IBM SPSS software package; SPSS Inc., released in 2008, produced the statistical analysis of the data. SPSS Statistics for Windows, Chicago: SPSS Inc.

3. Results

A total of 12 (55.5 %) and 10 (45.5 %) new cases were recorded in the QP and MBP, respectively. One patient (7.7 %) in the QP group had a relapse, in comparison with two patients in the MBP (16.7 %), (*p* = 0.571). Therefore, the total analysis was obtained from the evolution of 12 (48.0 %) episodes of TTP in the MBP, and 13 (53.0 %) episodes in the QP group.

3.1. Global sample description

The mean age was of 47 ± 18 years (n = 22); median = 46 years and the age ranged from five to 79 years. Sixteen patients were women (72.7 %). The TPE treatment was started the day of diagnosis or the day after (0.8 ± 3.5 days).

Within the total number of TPE recorded episodes (new diagnosis and relapses; n = 25), 14 episodes (56 %) presented anaemic syndrome, and 15 (60 %) reported Central Nervous System (CNS) involvement. Headache and migraine crisis were the most frequent CNS clinical findings, present in 40 % of these patients. Hemorrhagic diathesis (defined as any bleeding) was observed in 13 episodes (52 %) and other clinical manifestations in 16 (64 %); acute respiratory insufficiency (20 %) and abdominal pain (20 %), most frequently. One female patient was diagnosed during gestation; delivery was scheduled and TPE was not restarted because the patient had had a good response

to MBP treatment. The ADAMTS-13 activity was 5.9 % ± 10.5 % (n = 10) with neutralizing autoantibodies in five samples. Serum haptoglobin levels were determined in 14 episodes (56 %), where 10 (71.4 %) presented low values < 24 mg/dL. Direct Coombs test was negative, in all episodes (n = 18).

All episodes were treated with TPE. On average, patients required 13 ± 9 TPE processes, (median = 11 TPE), ranging from 1 to 41 TPE. The time from diagnosis to start of TPE was 1 ± 4 days on average, median = 0 days, ranged from −3 to 16 days. The TPE treatment lasted 19 ± 15 days, median = 17 days, ranged from one to 64 days. Most patients were given corticoids (84 %) per-protocol and, for some of them (28 %), other concomitant medication, usually Rituximab.

3.2. Treatment group's homogeneity

There was no statistical difference between the QP and MBP treatment groups concerning age (44 ± 20 years and 49 ± 15 years, respectively; *p* = 0.521) and gender (66.7 % and 80.0 % females, respectively; *p* = 0.646).

3.3. Comparative analysis of the episodes and processes

The episodes in both groups had similar clinical and laboratory data (Table 2). The MBP group had higher lymphocyte and monocyte counts than the QP group (*p* = 0.001 and *p* = 0.004, respectively).

No significant differences were observed in the mean number of TPE processes, days elapsed from diagnosis to start of TPE or total plasma

Table 2
Clinical, and laboratory testing in the diagnosis, by TPE group, based on the number of episodes.

	Plasma exchange group			
	QP (n = 13)	MBP (n = 12)	D	*p*-value
Clinical data				
Anemic Syndrome* (%)	46.2	66.7	−20.5	0.428
CNS involvement* (%)	76.9	41.7	35.2	0.111
Hemorrhagic diathesis* (%)	38.5	66.7	−28.2	0.238
Other clinical signs* (%)	69.2	58.3	10.9	0.688
Laboratory testing			D (CI 95%)	
Haemoglobin (gr/dL)	9.2 ± 2.9	8.3 ± 1.9	0.8 (−1.2; 2.8)	0.418
Haematocrit (%)	26.2 ± 8.3	23.5 ± 5.6	2.6 (−3.5; 8.8)	0.380
MCV (fL)	94.2 ± 8.4	91.1 ± 6.5	3.1 (−3.2; 9.4)	0.315
Platelets (x 10^9/L)	22.6 ± 26,7	14.6 ± 12,1	8.0 (−9.4; 25.5)	0.350
Leukocytes (x 10^9/L)	10.6 ± 3,7	8.3 ± 3.4	2.3 (−0.6; 5.3)	0.116
Segmented (%)	63.7 ± 18.1	62.8 ± 8.6	0.9 (−12.7; 14.5)	0.891
Lymphocytes (%)	9.7 ± 10,1	26.2 ± 6.5	−16.4 (−24.8; −8.0)	0.001
Monocytes (%)	3.6 ± 3.8	9.2 ± 3.7	−5.6 (−9.2; −2.0)	0.004
Schistocytes (%)	3.9 ± 2.1	3.6 ± 2.6	0.3 (−1.6; 2.3)	0.725
Reticulocytes (%)	8.0 ± 3.4	8.7 ± 5.3	−0.7 (−5.6; 4.2)	0.768
LDH (IU/L)	1,746 ± 1,047	1,732 ± 1,198	14 (−915; 943)	0.975
Urea (mg/dL)	85 ± 54	66 ± 36	19 (−26; 65)	0.386
Creatinine (mg/dL)	1.57 ± 0.93	1.35 ± 1.05	0.22 (−0.60; 1.04)	0.590
Bilirubin (mg/dL)	2.5 ± 1.33	3.65 ± 2.40	−1.15 (−2.88; 0.58)	0.179
Haemostasis				
PT (s)	89 ± 10	86 ± 10	2 (−7; 11)	0,620
aPTT (s)	31 ± 4	29 ± 2	2 (−1; 5)	0,101
Fibrinogen (mg/dL)	355 ± 100	438 ± 107	−83 (−170; 5)	0,063

* Values expressed on rates; otherwise expressed by mean ± SD.

Table 3
TPE-related parameters, by TPE group, based on the number of episodes and processes.

	Plasma exchange group			
Episodes	QP (n = 13)	MBP (n = 12)	D (CI 95 %)	*p*-value
TPE processes (n)	15 ± 12	10 ± 4	5 (−3; 12)	0.188
Diagnosis to treatment (days)	1.1 ± 4.9	0.5 ± 0.5	0.7 (−2.3; 3.6)	0.651
Days of treatment	24 ± 18	15 ± 8	9 (−3; 20)	0.004*
Total TPE volume (L)	44.0 ± 33.3	30.2 ± 13.0	13.8 (−7,5; 35,0)	0.193
Processes	QP (n)	MBP (n)	D (CI 95 %)	*p*-value
Volume/process (mL)	2,989 ± 795 (190)	3,046 ± 761 (119)	−37 (−217; 143)	0.684
Platelets (x 10^9/L)	12.6 ± 11.3 (189)	13.1 ± 9.5 (122)	0.5 (−2.9; 1.8)	0.642
LDH (IU/L)	667 ± 659 (161)	593 ± 888 (107)	−74 (−112; 261)	0.432

* Mantel-Cox survival analysis; Values expressed by mean ± SD.

volume transfused between the episodes of the QP and MBP groups (Table 3). From the survival analysis, the QP TPE was significantly associated with an increased time to recovery of TTP episodes compared with MBP treatment (*p* = 0.004). The estimated median days of treatment were 30 for the QP and 16 days for the MBP group. Besides, the estimated average of days of treatment was of 30 ± 5 days (CI: 19–40 days) in the QP group and 15 ± 2 days (CI: 11–19 days) in the MBP group.

In total, 193 TPE processes in the QP and 122 in the MBP took place during the period of evaluation in the two Centres. When comparing the TPE processes, although the parameters were not collected in all processes, there was no difference between the QP and the MBP groups in the transfused plasma volume, PLT count and LDH levels (Table 3).

3.4. Global survival rate

At the end of the evaluation period of the longitudinal analysis, of the 22 patients, 19 (86.4 %) were alive and free of disease with no disability (among them, 3 patients had suffered a successfully treated relapse), and three patients (13.6 %) had died. All deaths occurred in the QP group, although the difference with the MBP group was not significant (*p* = 0.221). The reported causes of death were a refractory shock (after one TPE session), irreversible coma (6 TPEs), and disseminated intravascular coagulation (DIC) plus acute myocardial infarction (11 TPEs).

Other characteristics (age, gender, clinical and laboratory data, concomitant medication and type of TPE treatment), showed no impact on survival between the groups of surviving and deceased patients. Patients who died had higher leucocyte levels (14.1 ± 4.7 × 10^9/L) when compared to the surviving patients (8.9 ± 3.2 × 10^9/L), (D = 5.2 × 10^9/L; *p* = 0.045; CI = 0.9–9.4 x 10^9/L). However, lymphocyte numbers were significantly higher in the surviving patients

(18.3 ± 11.3 %), than in deceased patients (1.9 ± 1.3 %), (D = 16.4%; *p* = 0.010; CI = 10.6–22.2 %).

Comparing the TPE days of treatment between living and deceased patients, non-significant differences were found between the patients who died (7 ± 5 days; n = 3) and those that survived (21 ± 15 days; n = 22; *p* = 0.106). However, none of the patients who died received TPE treatment for more than 11 days, whereas 72.7 % of those who survived were treated for a longer time (*p* = 0.037).

3.5. Comparative analysis of survival rate by TPE treatment processes

The comparative evaluation of the mean volume of plasma transfused by process resulted in significant difference between the patients who died (3,503 ± 1,059 mL; n = 18) and those that did not (2,994 ± 753 mL; n = 291). The PLT count was significantly lower in those patients who died (2.6 ± 3.1 × 10^9 PLT/L; n = 18) compared to those that did not (13.4 ± 10.6 × 10^9 PLT/L; n = 293), (D = 10.9 ± 1.0 × 10^9 PLT/L; *p* < 0.001; CI = 8.9–12.8 x 10^9 PLT/L), Further, the LDH level was significantly higher in those patients who died (1,785 ± 1,085 IU/L; n = 18) compared to those that did not (555 ± 659 IU/L; n = 250), (D = 1,230 ± 259 IU/L; *p* < 0.001; CI = 686–1,775 IU/L).

3.6. Analysis of surviving patients between TPE treatment groups

Because of the significant differences presented by the comparison of patients that survived and those patients who died during the evaluation period, a separate assessment was made of the subgroup of patients who survived.

From the 19 surviving patients group, none documented sequelae. Nine had been treated with QP (47.4 %) and 10 with MBP (52.6 %). Both groups were not significantly different regarding age (40 ± 19 and

Table 4
TPE-related parameters, by TPE group, based on the number of episodes and processes of surviving patients.

	Plasma exchange group			
Episodes	QP (n = 10)	MBP (n = 12)	D (CI 95 %)	*p*-value
TPE processes (n)	18 ± 12	10 ± 4	7 (−1; 16)	0.088
Days of treatment	29 ± 18	15 ± 8	14 (2; 26)	0.007*
Total TPE volume (L)	50.9 ± 34.1	30.2 ± 13.0	20.7 (−1.5; 42.8)	0.066
Processes	QP (n)	MBP (n)	D (CI 95 %)	*p*-value
Volume/process (mL)	2,957 ± 748 (172)	3,046 ± 761 (119)	−89 (−266; 88)	0.323
Platelets (x 10^9/L)	13.6 ± 11.4 (171)	13.1 ± 9.5 (122)	0.5 (−1.9; 2.9)	0.693
LDH (IU/L)	527 ± 413 (143)	593 ± 888 (107)	−66 (−232;100)	0.433

* Mantel-Cox survival analysis; Values expressed by mean ± SD.

49 ± 15 years, respectively; *p* = 0.259) or gender (77.8 % vs. 80.0 % females, respectively; *p* = 1.00). Anaemic syndrome, CNS involvement, hemorrhagic diathesis, and other clinical manifestations were not significantly different between groups either (data not shown). The need for concomitant medication was also not significantly different between the TPE groups.

Regarding clinical and laboratory data, the episodes in the treatment groups were homogeneous (data are not shown), except for the levels of lymphocytes and monocytes, which remained significantly higher in the group treated with MBP (*p* = 0.003 and *p* = 0.018, respectively).

No significant differences were observed in the mean number of TPE processes, days elapsed from diagnosis to start of TPE, and total plasma volume transfused between the episodes of the QP and MBP groups (Table 4). From the survival analysis, the QP TPE episodes were significantly associated with an increased time to recovery of TTP episodes compared with MBP treatment (*p* = 0.007). The estimated median days of treatment were 29 for the QP and 16 days for the MBP group. Besides, the estimated average of days of treatment was of 29 ± 6 days (CI: 18–40 days) in the QP group and 15 ± 2 days (CI: 11–19 days) in the MBP group. We detected that 60.0 % of episodes treated with QP took longer than 27 days while in contrast, none in the MBP group exceeded 27 days (*p* = 0.003).

By process, the transfused plasma volume, PLT count and LDH levels (Table 4) showed no statistical difference between the QP and MBP in the sub-group of patients that survived the acute TTP event during the period of study.

4. Discussion

We assessed the efficacy and safety of MBP pathogen-inactivated plasma compared with quarantine FFP for standard TPE in patients with acute TTP at two experienced Centres. Both of them followed the same TPE protocol. They were chosen for their geographical proximity and similar population characteristics, to avoid environmental or external differences between groups of patients and safeguard the reliability and external validity of these findings.

There were no significant differences between the TPE groups, in neither their demographic, clinical, biochemical, and haematological parameters, nor their therapeutic variables during the healing process. The leucocyte counts were similar, but the lymphocyte and monocyte counts were significantly higher in the MBP group only. This difference could show a different kind of immune response during the acute phases of TTP. ADAMTS13 deficiency is accepted as the main cause of idiopathic TTP [27], but other causes, such as lymphocytes and macrophages activation [28,29] have been suggested.

No differences were found regarding the TPE treatment between the two compared groups, even though the time to recovery was longer in the QP group. No impact on outcomes was found when a process -by-process evaluation showed that the TPE treatment did not prove to be different between the two groups, regarding plasma volume transfused, platelet count and LDH levels.

Regarding the remission rate, three patients died after a short period of QP treatment. These patients received more plasma volume, showed a lower PLT count and higher LDH levels at diagnosing than those who survived. No deaths were recorded in the MBP group. To avoid the bias due to the mortality observed in the QP group, which could influence the study results, we rerun the analysis culling the dead patients. No differences were found when comparing the processes between the two TPE treatment groups, but the QP patients still required more days of TPE treatment to recover compared to MBP treatment.

Our results dispute previous reports on the inferior efficacy and safety of MBP compared with QP. We believe this could arise from the populations studied and the statistical tools used.

One retrospective sequential study comparing QP and MBP in TTP patients [16] showed inferior results for MBP, in terms of the number of

TPE processes and duration of hospitalisation. The study design is unbalanced (QP group, n = 13; MBP group, n = 7) and collected during different periods (6 years, for the QP group and less than 3 years, for the MBP group). This kind of design has low statistical power and also reduces the likelihood that a statistically significant result reflects a true effect [30]. Besides, the TTP-related deaths happened between 5 and 8 days after diagnosis, in the QP group and 35 days, in the MBP group. Therefore, the QP group received fewer plasma units, during fewer episodes, and the hospitalisation time was much shorter. In small samples, extreme values may lead to a bias. However, results from the study did not reach statistical significance for the comparison of the number of TPE processes and duration of hospitalisation (*p* = 0.076 and *p* = 0.074, respectively), when removing from the analysis the three patients who died. Further, the complete remission rates (69.2 % and 57.1 %, in QP and MBP, respectively) were not significantly different (*p* = 0.651).

A retrospective study [17] reached similar conclusions. The study compared 56 TTP episodes from 43 patients, but the analysis approach is based on episodes only, a potential cause of misleading results. The age, gender, recurrence, and mortality rates we calculated taking into consideration the episodes, instead of patients. Within-subject serial dependence between episodes, recurrence, and mortality will result, with this method, misleading the interpretation of the data analysis. Besides, the *odds ratio* and beta-coefficients were not provided for the multivariate logistic regression model. Continuous data (days elapsed from starting TTP to the beginning of TPE, the volume of plasma replaced in the first 3 days), divided the subjects into categories. This way, the cutoffs tend to be arbitrary, and part of the information is lost [31]. Eventually, MBP was not inferior to QP in the total plasma volume exchange between the episodes (*p* = 0.9) and the remission rates showed no significant differences (*p* = 0.185).

A subsequent multicentre, prospective study [18] deduced that MBP is associated with a worse outcome in episodes of idiopathic TTP, based on the remission status on day 8 of treatment as the primary endpoint of the study, comparing 63 TTP episodes treated with MBP and 39 with QP. The study approach is based on episodes only, but the analysis equates episodes and patients, a potential cause of misleading results. The age, gender, remission, and mortality rates, we assessed considering the episodes, instead of patients, which is not coherent. Remission was defined as a response that eventually lasted for longer than 30 days after the withdrawal of TPE therapy, which is inconsistent with the primary endpoint. It is not clear the rationale of this definition, and no evidence-based explanation was given. Besides, no homogeneity analysis was carried out between centres, and the Mann-Whitney test used is not meaningful, given that the sample size was large enough in both TPE groups to use a parametric test [32].

Moreover, the variables were categorised arbitrarily. Irwin & McClelland [33] addresses the misconception that perhaps median splits are a good idea when the predictor variables are remarkably skewed, instead of the normal distributions. Even in those situations, splitting the data brings misleading results and confusions in the analysis of the results of a study. Likewise, the estimation of the logistic model requires basic assumptions to be met for logistic regression, including independence of errors, linearity in the logit for continuous variables, the absence of multicollinearity, and lack of strongly influential outliers [34,35], which are not mentioned in this prospective study. Besides, the resulting logistic regression model assessment using a goodness-of-fit measure is not shown. Before reaching definitive conclusions, the model's internal validity should formally quantify (i.e., replicability within the same data set) and external validity (i.e., generalizability beyond the current sample), which is not commented in their analysis. No survival analysis was found to evaluate the evolution of the patients. Eventually, remission was achieved in 97 % and 95 % episodes with MBP and QP, respectively. Notwithstanding, the response on day 8 of treatment was also assessed in this trial and found no significant difference between the MBP and QP group (58.3 % and 30.8 %,

respectively ($p = 0.238$).

There are some limitations to this study. The main one is that information was gathered retrospectively, and the number of events was small. While this shows the very low incidence of TTP, it also reduces confidence in the study findings. Conversely, we think the longitudinal analysis of these two different plasmas, largely used in routine in two different transfusion sites, may clear up the management of TTP and will help to strengthen the experience on these TPE alternatives.

Unfortunately, ADAMTS-13 activity was not available for all the patients, as the first-line treatment was frequently set up as an emergency based on clinical information only. However, the severe functional ADAMTS-13 deficiency requires a plasma quality with maintained levels of this enzyme to restore missing ADAMTS-13 activity in idiopathic TTP [36]. Several studies have shown that MBP retains similar levels when compared to fresh frozen plasma [12,15,37], maintaining its hemostatic properties.

Other virally inactivated plasmas, such as solvent/detergent (plasma pool), have been proven effective in TPE treatment. However because of its markedly reduced level of functional protein S activity, may predispose to the development of venous thrombosis, particularly after extensive, repeated TPE [14], in patients undergoing a liver transplant and patients with severe liver disease and known coagulopathies [38–40].

The scarce information about adverse events in the clinical records made it impossible to perform a thorough assessment of the incidence of adverse events (AE). The detected events were considered mild and did not require treatment interruption in most patients, except for one patient in the MBP group that presented a non-fatal acute pulmonary oedema. Some authors suggest the reduced risk of AE of MBP compared to QP, markedly in allergic reactions cases [41–44]. Thus, pathogen inactivated plasmas have been associated with fewer allergic reactions, febrile non-haemolytic reactions, transfusion circulatory overload (TACO) and hypotensive reactions than untreated plasma [45]. We have not found a physiopathological base for QP to be associated with a higher death rate, and this probably can only be ascertained by proteomic or metabolomic studies. Perhaps differences in plasma microvesicles between QP and MBP could play a role, as they have been shown to interfere with hemostasis [46].

In conclusion, this real-world study found that MBP and QP are effective and safe treatment strategies for idiopathic TTP patients. We found no difference between MBP and QP in terms of the number of TPE processes required, and the volume of total plasma transfused. Nevertheless, the findings of this study are favourable for patients who were treated with MBP since the QP TPE episodes were significantly associated with an increased number of days of treatment and safety concerns including the death risk. Furthermore, we consider it important to analyse the data carefully in the context and environment of this study.

Author contributions

José L. Arroyo Rodríguez and José M. García Gala designed the research study. Eva Martínez Revuelta and Cristina Amunárriz Águeda gathered the data and performed some of the TPE procedures. Carmen Muñoz Turrillas and Iñigo Romón Alonso provided technical assistance for blood collection and preparation. Ignacio Álvarez performed the statistical analysis. José Luis Arroyo Rodríguez and José María García Gala wrote the paper. All authors read and approved the final manuscript.

Funding

Declaration of Competing Interest

Acknowledgements

References

[1] Sadler JE. Von Willebrand factor, ADAMTS13, and thrombotic thrombocytopenic purpura. Blood 2008;112:11–8.

[2] Scott EA, Puca KE, Pietz BC, Duchateau BK, Friedman KD. Comparison and stability of ADAMTS13 activity in therapeutic plasma products. Transfusion 2007;47:120–5.

[3] Joly BS, Coppo P, Veyradier A. Thrombotic thrombocytopenic purpura. Blood 2017;129:2836–46.

[4] Mollison's blood transfusion in clinical medicine. 12 ed. Oxford: Wiley Blackwell; 2014.

[5] Rock GA, Shumak KH, Buskard NA, Blanchette VS, Kelton JG, Nair RC, et al. Comparison of plasma exchange with plasma infusion in the treatment of thrombotic thrombocytopenic purpura. Canadian apheresis study group. N Engl J Med 1991;325:393–7.

[6] Alwan F, Vendramin C, Vanhoorelbeke K, Langley K, McDonald V, Austin S, et al. Presenting ADAMTS13 antibody and antigen levels predict prognosis in immune-mediated thrombotic thrombocytopenic purpura. Blood 2017;130:466–71.

[7] Schwartz J, Padmanabhan A, Aqui N, Balogun RA, Connelly-Smith L, Delaney M, et al. Guidelines on the use of therapeutic apheresis in clinical practice-evidence-based approach from the writing committee of the american society for apheresis: the seventh special issue. J Clin Apher 2016;31:149–62.

[8] European Directorate for the Quality of Medicines & HealthCare. Guide to the preparation, use and quality assurance of blood components Recommendation No. R (95) 15. 18th ed. Council of Europe; 2015.

[9] Seghatchian J, Walker WH, Reichenberg S. Updates on pathogen inactivation of plasma using Theraflex methylene blue system. Transfus Apher Sci 2008;38:271–80.

[10] Heger A, Kannicht C, Romisch J, Svae TE. Normal levels of ADAMTS13 and factor H are present in the pharmaceutically licensed plasma for transfusion (Octaplas) and in the universally applicable plasma (Uniplas) in development. Vox Sang 2007;92:206–12.

[11] Mintz PD, Neff A, MacKenzie M, Goodnough LT, Hillyer C, Kessler C, et al. A randomized, controlled phase III trial of therapeutic plasma exchange with fresh-frozen plasma (FFP) prepared with amotosalen and ultraviolet A light compared to untreated FFP in thrombotic thrombocytopenic purpura. Transfusion 2006;46:1693–704.

[12] Cardigan R, Allford S, Williamson L. Levels of von Willebrand factor-cleaving protease are normal in methylene blue-treated fresh-frozen plasma. Br J Haematol 2002;117:253–4.

[13] Osselaer JC, Debry C, Goffaux M, Pineau J, Calomme G, Dubuc E, et al. Coagulation function in fresh-frozen plasma prepared with two photochemical treatment methods: methylene blue and amotosalen. Transfusion 2008;48:108–17.

[14] Hacquard M, Lecompte T, Belcour B, Geschier C, Jacquot C, Jacquot E, et al. Evaluation of the hemostatic potential including thrombin generation of three different therapeutic pathogen-reduced plasmas. Vox Sang 2011;102:354–61.

[15] Yarranton H, Lawrie AS, Purdy G, Mackie IJ, Machin SJ. Comparison of von Willebrand factor antigen, von Willebrand factor-cleaving protease and protein S in blood components used for treatment of thrombotic thrombocytopenic purpura. Transfus Med 2004;14:39–44.

[16] de la Rubia J, Arriaga F, Linares D, Larrea L, Carpio N, et al. Role of methylene blue-treated or fresh-frozen plasma in the response to plasma exchange in patients with thrombotic thrombocytopenic purpura. Br J Haematol 2001;114:721–3.

[17] Alvarez-Larran A, Del Rio J, Ramirez C, Albo C, Pena F, et al. Methylene blue-photoinactivated plasma vs. fresh-frozen plasma as replacement fluid for plasma exchange in thrombotic thrombocytopenic purpura. Vox Sang 2004;86:246–51.

[18] del Rio-Garma J, Alvarez-Larran A, Martinez C, Muncunill J, et al. Methylene blue-photoinactivated plasma versus quarantine fresh frozen plasma in thrombotic thrombocytopenic purpura: a multicentric, prospective cohort study. Br J Haematol 2008;143:39–45.

[19] Williamson LM, Cardigan R, Prowse CV. Methylene blue-treated fresh-frozen plasma: what is its contribution to blood safety? Transfusion 2003;43:1322–9.

[20] Seghatchian J, Struff WG, Reichenberg S. Main properties of the THERAFLEX MB-Plasma system for pathogen reduction. Transfus Med Hemother 2011;38:55–64.

[21] Mohr H, Knuver-Hopf J, Gravemann U, Redecker-Klein A, Muller TH. West Nile virus in plasma is highly sensitive to methylene blue-light treatment. Transfusion 2004;44:886–90.

[22] Fryk JJ, Marks DC, Hobson-Peters J, Prow NA, Watterson D, Hall RA, et al. Dengue and chikungunya viruses in plasma are effectively inactivated after treatment with methylene blue and visible light. Transfusion 2016;56:2278–85.

[23] Fryk JJ, Marks DC, Hobson-Peters J, Watterson D, Hall RA, Young PR, et al.

Reduction of Zika virus infectivity in platelet concentrates after treatment with ultraviolet C light and in plasma after treatment with methylene blue and visible light. Transfusion 2017;57:2677–82.

[24] Eickmann M, Gravemann U, Handke W, Tolksdorf F, Reichenberg S, Muller TH, et al. Inactivation of Ebola virus and middle east respiratory syndrome coronavirus in platelet concentrates and plasma by ultraviolet C light and methylene blue plus visible light, respectively. Transfusion 2018;58:2202–7.

[25] Eickmann M, Gravemann U, Handke W, Tolksdorf F, Reichenberg S, Müller TH, et al. Inactivation of three emerging viruses - severe acute respiratory syndrome coronavirus, Crimean-Congo haemorrhagic fever virus and Nipah virus - in platelet concentrates by ultraviolet C light and in plasma by methylene blue plus visible light. Vox Sang 2020. https://doi.org/10.1111/vox.12888.

[26] Reichenberg S, Gravemann U, Sumian C, Seltsam A. Challenge study of the pathogen reduction capacity of the THERAFLEX MB-Plasma technology. Vox Sang 2015;109:129–37.

[27] Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. N Engl J Med 1998;339:1585–94.

[28] Neame PB. Immunologic and other factors in thrombotic thrombocytopenic purpura (TTP). Seminars in Thrombosis and Hemostasis 6. 1980. p. 416–29.

[29] Wada H, Kaneko T, Ohiwa M, Tanigawa M, Tamaki S, Minami N, et al. Plasma cytokine levels in thrombotic thrombocytopenic purpura. Am J Hematol 1992;40:167–70.

[30] Button KS, Ioannidis JP, Mokrysz C, Nosek BA, Flint J, Robinson ES, et al. Power failure: why small sample size undermines the reliability of neuroscience. Nat Rev Neurosci 2013;14:365–76.

[31] Ranganathan P, Pramesh CS, Aggarwal R. Common pitfalls in statistical analysis: logistic regression. Perspect Clin Res 2017;8:148–51.

[32] Armitage P, Berry G, Matthews JNS. Statistical methods in medical research. 2ed Blackwell; 1987.

[33] Irwin JR, McClelland GH. Negative consequences of dichotomizing continuous predictor variables. J Mark Res 2003;40:366–71.

[34] Hosmer DW, Lemeshow S. Applied logistic regression. New York: Wiley; 1989.

[35] Kleinbaum DG. Logistic regression: a self-learning text 2ed. Springer; 1994.

[36] Kiss JE, Uhl L. Telltale signs of progress in the management of thrombotic thrombocytopenic purpura. Transfusion 2012;52:2498–501.

[37] del Rio-Garma J, Pereira A, Arroyo JL, Mateo J, et al. ADAMTS-13 activity and von Willebrand factor levels in methylene-blue photo-inactivated plasma processed by either the Springe method or an' in house' system. Vox Sang 2008;95:101–5.

[38] Flamholz R, Jeon HR, Baron JM, Baron BW. Study of three patients with thrombotic thrombocytopenic purpura exchanged with solvent/detergent-treated plasma: is its decreased protein S activity clinically related to their development of deep venous thromboses? J Clin Apher 2000;15:169–72.

[39] Yarranton H, Cohen H, Pavord SR, Benjamin S, Hagger D, Machin SJ. Venous thromboembolism associated with the management of acute thrombotic thrombo-cytopenic purpura. Br J Haematol 2003;121:778–85.

[40] Alvarez I, Reichenberg S, di Nicola S. Plasma transfusion volume and liver trans-plantation safety. Transfusion 2013;53:2353–4.

[41] Nussbaumer W, Mayersbach P, Gächter E, Schennach H, Mayer W. Reduced number of adverse events with methylene-blue treated plasma compared with quarantine stored plasma: a single center's experience. Vox Sang 2012;103:1–271.

[42] Nussbaumer W, Mayer W, Schennach H, Astl M. Missing influence of plasma quality (quarantine-stored and methylene blue-treated) on the frequency of side effects and the consumption of red blood cells and platelet units. Transfusion Anaheim 2015.

[43] Politis C, Kavallierou L, Hantziara S, Parara M, Zervou E, Katsarou O, et al. Haemovigilance data on the use of methylene blue virally inactivated fresh frozen plasma with the Theraflex MB-plasma system in comparison to quarantine plasma: 11 years' experience. Transfus Med 2014;24:316–20.

[44] Saadah NH, van Hout FMA, Schipperus MR, le Cessie S, et al. Comparing transfusion reaction rates for various plasma types: a systematic review and meta-analysis/regression. Transfusion 2017;57:2104–14.

[45] Saadah NH, van der Bom JG, Wiersum-Osselton JC, Richardson C, Middelburg RA, et al. Comparing transfusion reaction risks for various plasma products - an analysis of 7 years of ISTARE haemovigilance data. Br J Haematol 2018;180:727–34.

[46] Ridger VC, Boulanger CM, Angelillo-Scherrer A, Badimon L, Blanc-Brude O, et al. Microvesicles in vascular homeostasis and diseases. Position paper of the European society of cardiology (ESC) working group on atherosclerosis and vascular biology. Thromb Haemost 2017;117:1296–316.