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ORIGINAL PAPER

# International, prospective haemovigilance study on methylene blue-treated plasma

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**Background and objectives** Methylene blue is a phenothiazine dye, which in combination with visible light has virucidal and bactericidal properties, disrupting the replication of a broad range of enveloped viruses and some non-enveloped viruses. The study objective was to collect data on adverse reactions occurring with methylene blue plasma administered in a routine clinical practice environment and document their characteristics and severity.

**Materials and methods** This was an open label, multicentre, non-controlled, non-randomized, non-interventional study. Patients who receive a methylene blue plasma transfusion were observed for any signs and symptoms (adverse reactions) within 24 h safter the start of the transfusion, in different hospitals for a study duration of at least 1 year.

**Results** A total of 19 315 methylene blue plasma units were transfused. There were eight patients with adverse reactions recorded during the study, one of them serious. Two had more than one reaction (two and four, respectively). Three patients had previous transfusions with methylene blue plasma only.

**Conclusion** Methylene blue plasma has a very acceptable safety profile with a rate of serious adverse reactions of 0·5/10 000 units.

**Key words:** blood safety, haemovigilance, methylene blue, pathogen reduction.

## Introduction

The implementation of pathogen reduction (PR) for labile blood components aims at reducing a potential infection risks mainly by emerging pathogens.

The technology to use methylene blue (MB) for the PR of plasma for therapeutic use was first developed by the Blood Centre of the German Red Cross, Springe, Germany, and has been used in clinical practice for 20 years [1]. MB is a phenothiazine dye, which, in combination with visible light, has virucidal properties by interacting with nucleic acids and disrupting the replication of a broad range of enveloped viruses and some non-enveloped viruses [2] as well as other micro-organisms.

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MacoPharma improved the original Springe method by developing the THERAFLEX MB-Plasma system [3], marketed in several countries worldwide as a PR system for plasma to reduce the risk of transmission of pathogens. The system comprises a disposable kit incorporating MB and partially an MB removal filter (BLUEFLEX) as well as an illumination device for the application of the visible light.

In 2011, the French national drug regulatory agency ANSM (previously known as AFSSAPS) decided to progressively stop the clinical use of MB-treated plasma. One reason for this decision was a reported higher risk of severe allergic reactions for patients transfused with MB-treated plasma as compared to patients transfused with plasma treated with other PR methods [4]. In contrast, this higher risk has not been observed in other countries even in those not using the BLUEFLEX plasma filter after illumination; however, the available data come from their

national haemovigilance reports, using different methodologies and based on voluntary communication only. A prospective and exhaustive haemovigilance approach might help to clarify this issue.

Therefore, a non-interventional study was performed where patients, who were transfused with plasma prepared with the THERAFLEX MB-Plasma system, were observed according to local standard clinical practice. This study aimed to collect data on the frequency and nature of transfusion reactions following transfusion of MB plasma administered in a routine clinical practice environment and to learn more about their characteristics and about the possible factors that may have influenced their presentation and evolution.

## Materials and methods

The study was designed as an open label, CRO-managed, multicentre, non-controlled, non-randomized, non-interventional study, to evaluate the safety of MB plasma over a minimum period of 1 year, starting in May 2013. The end date of the planned study period was extended until April 2015 to allow each study centre a total study observation period of 1 year. The study was performed at four blood centres in Spain (Axencia Galega de Sangue, Órganos e Tecidos, Santiago de Compostela), United Kingdom (NHSBT Borehamwood, Bristol and Manchester), Belgium (Universitair Ziekenhuis, Ghent) and Greece (General Hospital of Athens Evaggelismos, Athens), supplying seven hospitals.

The study defined an adverse drug reaction as a response to a drug which is noxious and unintended and which occurs at doses normally used for prophylaxis, diagnosis or therapy of disease or for modification of physiological function (ICH E2A, II/A/2 [5]). Additionally, the protocol followed the International Society of Blood Transfusion (ISBT) definition of an adverse reaction (AR) as an undesirable response or effect in a subject temporally associated with the administration of blood or blood component [6].

Plasma was obtained from local donors in each country, except in the United Kingdom, where the Departments of Health have recommended that the FFP given to neonates and children born after 1 January 1996 should be obtained from an area free of bovine spongiform encephalopathy and subjected to pathogen reduction procedures [7]. Following these guidelines, the plasma was produced in Austria.

The photodynamic procedure using methylene blue (MB) and visible light is applied to single donor units of plasma only. MB in whole blood, after i.v. administration, has an estimated terminal half-life of 5·25 h [8]. However, all patients, who received at least one MB-plasma transfusion at the study centres, were monitored for the occurrence of possible acute AR for 24 h after start of the transfusion following the definition of the British committee for standards in haematology [9] and ISBT [6] and this monitoring was performed and recorded according to their predefined local methodology. The haemovigilance Case Report Form (CRF) was completed for all patients who had received an MB-plasma transfusion and experienced an AR. The subjects were identified by a number and/or letter code. The patient informed consent was obtained at the time of the occurrence of the AR. All data were captured via electronic data capture (EDC) using a web-based tool. The software Marvin from the company XClinical (www.xclinical.com/) was the preferred EDC software. Marvin is compliant with all legislation relevant to electronic data capture: US Food and Drug Administration (FDA) 21 Code of Federal Regulations (CFR), part 11 [10], International Conference on Harmonization – Good Clinical Practice (ICH-GCP) [11]. Assignment of a particular blood component to a subject was not decided in advance by the study protocol, but fell within current practice.

Details about the type of transfusion, transfused units, volume and other non-plasma blood components transfused during the same episode were captured and documented. Details about the plasma source (collection from whole blood, aphaeresis) and reference number of the MB kit used were also collected, as well as information about whether or not the BLUEFLEX MB removal filter was used, since the residual MB is not routinely removed in Spain, and the respective illumination device (Macotronic B2 or V4).

Demographic data (age and gender), information about the diagnosis and indication, the transfusion order and execution and any history of previous transfusion reactions were recorded in the haemovigilance CRF.

Subjects who received an MB-plasma transfusion were observed for any signs and symptoms within 24 h after the start of transfusion by each medical team incharge of the patient's care, which was thoroughly informed before the study starting, which in turn, informed the research team of an adverse reaction as soon as it happened. In the UK, due to hospital requirements, a trained nurse was devoted to monitoring the study throughout its duration. If the subject experienced an AR, the date and time were recorded, the subject's signs and symptoms were documented in the haemovigilance CRF by ticking all signs and symptoms that applied from a predefined list (i.e. more than one sign or symptom could be reported) or, if not included in the list, by specifying the signs or symptoms.

The following predefined signs and symptoms, commonly observed during or after a transfusion, were listed

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**Table 1** Diagnosis of adverse reactions and Medical Dictionary for Regulatory Activities (MEDDRA v.18.0, MeDRA-R, IFPMA on behalf of ICH, McLean, VA, USA) preferred term (PT)

| Subject no. | MedDRA |
|---|---|
| All subjects | Allergic transfusion reaction/anaphylactic transfusion reaction |
| | Acute haemolytic transfusion reaction |
| | Delayed haemolytic transfusion reaction |
| | Delayed serologic transfusion reaction |
| 21-003 | Febrile non-haemolytic transfusion reaction |
| 21-002; 41-001 | Hypotensive transfusion reaction |
| | Viral infection/transmission of an infectious agent via product |
| | Bacterial infection/transmission of an infectious agent via product |
| | Posttransfusion purpura |
| | Transfusion-related circulatory overload |
| 41-002 | Transfusion-associated dyspnoea |
| | Transfusion-associated graft-*versus*-host disease |
| | Transfusion-related acute lung injury |

in the haemovigilance CRF: oedema, flushing, rash, pruritus, urticaria, abdominal pain, infusion site pain, chills/rigours, fever, nausea/vomiting, tachycardia, hypotension, shock, dyspnoea and bronchospasm. Physicians allocated the signs and symptoms to one of the diagnoses as listed in Table 1. With the exception of infections, these diagnoses are defined in the 'proposed standard definitions for surveillance of non-infectious adverse transfusion reactions' [6]. If not included in this list, the physician was required to specify his/her diagnosis or check 'unknown pathophysiology'.

AR were classified as either serious or non-serious. A serious adverse transfusion reaction (SATR) is any untoward medical occurrence that results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity or is a congenital anomaly/birth defect [5]. Besides, all occurring AR were graded with respect to severity, and their imputability was assessed. Severity was rated as Non-severe, Severe, Life-threatening, Death or Unknown. Definitions of all imputability levels are provided in Table 2. Imputability level 1 stands for either 'unlikely' or 'possible'. Only AR with imputability levels 1 3 were recorded in this study.

To be eligible for participation in the study, the subjects had to fulfil the following criteria: (1) written informed consent and (2) subjects who had experienced an AR after receiving a transfusion with MB plasma produced using the THERAFLEX MB-Plasma procedure from MacoPharma. Subjects who received a transfusion with another plasma type during the same transfusion episode were excluded from study participation.

The primary end-points were the annualized incidence of adverse transfusion reactions (ATR) following administration of MB plasma based on the total number of transfused units administered and transfusion episodes.

The secondary end-points were as follows: (1) the incidence of specific ATR, following administration of MB plasma and (2) the potential relationship between ATR and the different parameters recorded.

If a subject experienced an AR, the physician had to initiate appropriate treatment according to his/her medical judgment and practice. Subjects who experienced an AR were followed up for 28 days after the occurrence of the AR by additional examinations according to the medical practice of the investigator. Additionally, the total number of MB-plasma transfusions, the number of recipients and the number of units administered in each study centre were collected. The centres were monitored by trained clinical research associates visiting the trial sites on a monthly basis.

## Statistical analysis

For the analysis of the primary end-points, the incidence rate was calculated using the number of transfused units with at least one transfusion reaction based on the total number of transfused units and the number of transfusions with at least one transfusion reaction based on the number of transfusions administered during the observation period.

The annualized rate was calculated as

$$\text{Annualized transfusion reaction rate} = \frac{\text{No. of transfused units/transfusion episodes with at least one transfusion reaction over the whole observation period} \times 365{\cdot}24}{\text{Duration of observational period [days]}}$$

Duration of observational period: difference between date of inclusion of the study centre and date of end of the observation period.

Frequency tabulation showing the number of ATR with counts and percentages based on all transfused units and on all transfusions were prepared. The number of all transfusions and transfused units were gathered every 3 months from the different transfusion centres. Only the related ATR (possible, likely/probable, certain related) were used for the analysis and 95% confidence intervals were provided.

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**Table 2** Imputability levels to assess adverse events (Commission Directive 2005/61/EC [21])

| Imputability level | | Explanation |
|---|---|---|
| NA | Not assessable | When there is insufficient data for imputability assessment. |
| 0 | Excluded | When there is conclusive evidence beyond reasonable doubt for attributing the adverse event to alternative causes. |
| 1 | Unlikely | When the evidence is clearly in favour of attributing the adverse event to causes other than the blood or blood components. |
| | Possible | When the evidence is indeterminate for attributing adverse event either to the blood or blood component or to alternative causes. |
| 2 | Likely, probable | When the evidence is clearly in favour of attributing the adverse event to the blood or blood component. |
| 3 | Certain | When there is conclusive evidence beyond reasonable doubt for attributing the adverse event to the blood or blood component. |

Incidence of specific transfusion reactions following administration of MB plasma was analysed and tabulated as for the primary end-point with stratification of the incidence rates by MedDRA coded diagnosis of ATR.

Summary tables based on the number of transfusions, number of units transfused and number of recipients were prepared showing the number and percentage of events including a 95% confidence limit for the following selections: ATR, SATR and SATR leading to death as outcome. If a subject had more than one ATR which coded to the same PT, the subject was counted only once for that PT.

## Results

Eight subjects of 3005 observed patients experienced an ATR after transfusion with MB plasma and were included in this study, five subjects in Spain and three subjects in Belgium. One of the subjects experienced a life-threatening SATR. No ATR were reported at the study centres in the UK or Greece.

As shown in Table 3, the total number of units of MB plasma transfused treated with the THERAFLEX MB-Plasma procedure was 19 315 units and the total number of transfusions administered was 3780. A total of 3005 subjects received transfusions with MB plasma during the observation period (ranged from 365 to 626 days). The total number of 'observation days' was 1865 days. The mean age of the subjects was 45·8 years (standard deviation [SD]: 25·16) with an age range between 11 and 78 years. Five subjects were male, and three subjects were female. Detailed subjects characteristics and the date of inclusion are listed in Table 4. No history of allergies was reported in seven subjects; one subject (41-002) had a latex allergy. Only three of the eight subjects had received prior transfusions with MB plasma with no transfusion reaction reported: subject 21-004 received plasmapheresis for thrombotic thrombocytopenic purpura; subject 41-001 received a plasma transfusion during surgery; subject 41-002 required a plasma transfusion due to isolated shortage of coagulation factor V or XI. None of the eight subjects had prior transfusions with other types of plasma. In most cases (five subjects; 62·5%), haematological disease was the primary indication for the transfusion. Two subjects received a transfusion during surgery and one subject required a transfusion due to 'digestive bleeding'.

Subject 21-001 received between 12 and 14 units of MB plasma daily over four days and an ATR was reported for each day. Subject 21-004 received a total of 23 units of MB plasma over two days, and experienced an ATR on both days. All other subjects experienced one ATR after one transfused unit of MB plasma.

ATR were reported for 12 transfusions of MB plasma, which were administered to the eight subjects. Details of the transfusions are provided in Table 5. The total number of units transfused was 92 units, and per subject

**Table 3** Study overview by country. Number of THERAFLEX MB-Plasma units

| | Spain | UK | Belgium | Greece | Total |
|---|---|---|---|---|---|
| Number of units transfused | 5952 | 434 | 3688 | 9241 | 19 315 |
| Number of transfusions | 1067 | 378 | 1082 | 1253 | 3780 |
| Number of subjects with transfusions | 804 | 168 | 799 | 1234 | 3005 |
| Duration of observation period (days) | 626 | 381 | 493 | 365 | 1865 |
| Rate of ATR per 1000 units transfused | 1·51 | 0 | 0·81 | 0 | 0·62 |
| Rate of ATR per 100 patient transfused | 0·50 | 0 | 0·38 | 0 | 0·04 |

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**Table 4** Subject characteristics

| Subject no. | Date of inclusion | Age at inclusion (years) | Gender | Blood type and Rh factor | Previous with MB-plasma transfusion | Country[a] |
|---|---|---|---|---|---|---|
| 21-001 | 07 Nov 2013 | 29 | Male | AB negative | – | Spain |
| 21-002 | 04 Dec 2013 | 78 | Female | A positive | – | Spain |
| 21-003 | 19 Nov 2013 | 47 | Male | O positive | – | Spain |
| 21-004 | 15 Apr 2014 | 53 | Female | O positive | Yes | Spain |
| 21-005 | 05 Mar 2015 | 78 | Male | O positive | – | Spain |
| 41-001 | 15 Jan 2014 | 52 | Male | O positive | Yes | Belgium |
| 41-002 | 13 Aug 2014 | 11 | Male | B positive | Yes | Belgium |
| 41-003 | 01 Sep 2014 | 18 | Female | O positive | – | Belgium |

[a]Country where the transfusion was administered.

**Table 5** Details of transfusions that caused adverse transfusion reactions

| Subject no. | Primary indication | Date of transfusion | Transfused | | Plasma source | Blueflex filter used | Signs and symptoms |
|---|---|---|---|---|---|---|---|
| | | | Volume (ml) | Units | | | |
| 21-001 | Haematological | 06 Nov 2013 | 2800 | 12 | WB | No | Rash, pruritus, urticaria |
| | | 07 Nov 2013 | 2800 | 12 | WB | Yes | Rash, pruritus, urticaria |
| | | 08 Nov 2013 | 3000 | 13 | WB | Yes | Rash, pruritus, urticaria |
| | | 09 Nov 2013 | 3200 | 14 | WB | Yes | Rash, pruritus, urticaria |
| 21-002 | Surgery | 03 Dec 2013 | 273 | 1 | Aph | No | Rash, pruritus, fever, hypotension, shock |
| 21-003 | Surgery | 16 Nov 2013 | 229 | 2 | WB | No | Pruritus, fever |
| 21-004 | Haematological | 15 Apr 2014 | 2267 | 11 | Aph | No | Pruritus, urticaria |
| | | 16 Apr 2014 | 2798 | 12 | WB | Yes | Urticaria |
| 21-005 | Digestive bleeding | 04 Mar 2015 | 257 | 1 | WB | No | Rash, pruritus, urticaria |
| 41-001 | Haematological | 13 Jan 2014 | 213 | 1 | Aph | Yes | Flushing, pruritus, urticaria, hypotension |
| 41-002 | Haematological | 11 Aug 2014 | 232 | 9 | Aph | Yes | Rash, dyspnoea |
| 41-003 | Haematological | 19 Aug 2014 | 256 | 4 | Aph | Yes | Urticaria |

WB, whole blood; Aph, aphaeresis.

ranged from 1 unit to 14 units per day. The BLUEFLEX filter was used for seven of the 12 transfusions (58·3%). The V4 illumination device model was used in Spain, UK and Belgium, while the B2 illumination device was in use in Greece. The annualized rate incident for ATRs was based on the number of transfusions with at least one AR and was the same as for transfusion units. For the calculation, it was assumed that per transfusion only one transfusion unit caused the ATR regardless whether one or more units were given per transfusion. Based on the total number of units transfused during the observational period (19 315 units), the incidence rate for ATR ($n$ = 12) was 0·06% (95% confidence interval [CI]: 0·032–0·108. Based on the total number of transfusion episodes ($n$ = 3780), the incidence of ATR was 0·32% (95% CI 0·164 0·554).

Overall, eight (0·27%) of a total of 3005 subjects who received a transfusion with MB plasma, experienced at least one ATR (95% CI: 0·115–0·524); 58% of plasma units with registered ATR were derived from whole blood and 42% from aphaeresis. Nine of them were considered non-severe, and only one of the three severe ATR was graded as life-threatening.

The majority of the subjects who experienced an ATR after transfusion of MB plasma (7/8 subjects, 87·5%) had an allergic reaction with one or more of the following symptoms: rash, pruritus, urticaria, fever, flushing. In total, 12 allergic transfusion reactions were reported in these seven subjects. Other ATR reported were anaphylactic transfusion reaction (one subject), hypotensive transfusion reaction (one subject) and transfusion-associated dyspnoea (1 subject). The imputability was rated as 'Certain' in five ATR, 'Probable' in four and 'Possible' in three; 75% of the ATR required antihistamine treatment and 25% antihistamine and corticoids. All subjects completely recovered, most of them in less than an hour.

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Only one severe and SATR was observed throughout the study. The recipient (subject 21-002), a 78-year-old female, underwent surgery and received also platelet concentrates and red blood cells during the intervention. The patient experienced a life-threatening anaphylactic transfusion reaction 5 min after the start of one aphaeresis MB-plasma transfusion during surgery. The subject developed a rash, pruritus, fever and hypotension and subsequently shock. She was treated with antihistamine and corticoids and the symptoms resolved in 20 min. The transfused volume of MB plasma was 273 ml, and the Blueflex filter was not used. No history of allergies or previous transfusions was reported. The imputability was considered as 'Possible' (Grade 1). The subject received only one unit during the study period.

It was not possible to address the potential relationship between ATR and the different parameters recorded due to the low number of ATR registered.

## Discussion

This study aimed to collect detailed prospective haemovigilance data from different centres, from different countries, in which MB-plasma pathogen reduction technology is used routinely. The information should be used to reconcile the higher than expected observed frequency of serious adverse reaction notifications occurring during transfusions of MB plasma in France between 2008 and 2012 period (published by ANSM) and the (contradictory) lower incidences also reported retrospectively by other authors, in France or other countries (Table 6). In fact, the incidence rates of SATR range from 0 to 1 per 10 000 transfused units, small differences without statistical relevance.

In the current study, the incidence of SATR was 1 per 19 315 transfused units of MB plasma, or 0·5/10 000 transfused units, higher than the rate showed in a previous big European retrospective study [12]. Besides, it was similar to the incidence published from the French data base e-fit V3 [13], for 2009, 2010 (0·6/10 000 units, each year) and 2011 (0·5/10 000 units). However, it was lower than the frequency registered in 2008 (1·0/10 000 units), when the MB-plasma pathogen reduction system implementation started at midyear, but higher than the 2012 rate (0·3/10 000 units).

It is noteworthy that an extended data base record collecting regional data in France registered one SATR only [14], between 2008 and 2011 years, and no one was recorded in other countries [14, 15], matching the same outcome as registered in Greece and the United Kingdom, during our study.

**Table 6** Published rates of adverse transfusion reactions, and serious adverse transfusion reactions, related to MB plasma

| Country | Year | Total units | SATR/10 000 |
|---|---|---|---|
| Austria, Belgium, France, Greece, Spain, UK [12] | 2007–2011 | 1 547 105 | 0·2 |
| France (ANSM) [13] | 2008–2012 | 750 564 | 0·3–1·0 |
| France [14] | 2000–2011 | 10 283 | 1·0 |
| Greece [15] | 2001–2011 | 132 325 | 0 |
| Austria [16] | 2009–2014 | 23 920 | 0 |
| United Kingdom [17, 20] | 2012–2013 | 29 441 | 0·6–0·7 |
| Belgium [18, 19] | 2012–2013 | 150 322 | 0·2–1·0 |
| Belgium, Greece, Spain and UK (this study) | 2013–2015 | 19 315 | 0·5 |

**Table 7** Published rates of adverse transfusion reactions, and serious adverse transfusion reactions, related to non-MB-treated plasmas

| Country | Year | System | Total Units | SATR/10 000 |
|---|---|---|---|---|
| France (ANSM) [13] | 2008–2012 | Quarantine | 265 460 | 0·1–0·3 |
| | | Amotosalen | 298 856 | 0·3–0·8 |
| | | Solvent-Detergent | 535 490 | 0·3–0·5 |
| France [14] | 2000–2011 | Quarantine | 40 631 | 0·2 |
| | | Amotosalen | 36 035 | 0·6 |
| | | Solvent-Detergent | 19 015 | 0·0 |
| Greece [15] | 2001–2011 | Quarantine | 314 895 | 0·7 |
| Austria [16] | 2009–2014 | Quarantine | 32 217 | 0·2 |
| United Kingdom [17, 20] | 2012–2013 | Quarantine | 549 053 | 1·0–1·1 |
| United Kingdom [17, 20] | 2012–2013 | Solvent-Detergent | 157 142 | 0·1–0·3 |

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Other official publications from UK [16, 17] and Belgium [18, 19], and reporting 2012 and 2013 haemovigilance data, a period not covered by the previously mentioned studies, found quite similar results, documenting the safety profile of the MB-plasma pathogen reduction system used.

Unfortunately, due to the differences in methodology such as in recording the ATR and their imputability, an attempt to a meta-analysis was considered not feasible.

Adverse transfusion reactions due to plasma transfusion are rather common. Most of these are allergic/anaphylactic ATR. The decision of ANSM was based on the reported higher incidence rate of MB plasma relative to other plasma types. However, compared to other plasma types (Table 7), the published data showed similar rates in France [13, 14] with quarantine plasma, as those obtained with MB plasma in our study. On the other hand, the incidences with quarantine plasma were significantly higher in Greece [15] and Austria [16], compared to those observed with MB plasma in these countries. Analogous results have been communicated with amotosalen-plasma pathogen reduction system in France [13, 14] and slightly lower rates with the solvent-detergent-plasma procedure in France and UK [13, 14, 17, 20].

Additionally, it is worth to note that the BLUEFLEX plasma filter is not used in routine after illumination in Spain and yet, the rate of SATR in our study and historically [12] is extremely low. Even though an apparent relationship with the BLUEFLEX plasma filter has not been shown, its use is being reasonably considered by several transfusion centres of Spain, to reduce potential allergic reactions, especially in children.

In our opinion, the rates of ATR and SATR observed in this study confirm those incidences observed with the MB-plasma pathogen reduction system, by different authors, in several European countries. The significant number of units transfused up to date, and the large accumulated experience, makes these incidences consistent enough to conclude or consider that a substantial change will not occur in time.

The limitations of the study included the risk of investigator reporting bias as a result of the open label, non-controlled design with no comparator. Furthermore, the SATR was not confirmed by skin testing because the imputability was graded 1, as the patient received other blood components during the same transfusion episode. On the other hand, the low rate of ATR prevented any association analysis between ATR characteristics and other different parameters recorded.

We consider this study particularly relevant since most of the haemovigilance studies gather retrospective data. Sources of error due to confounding and bias are more common in retrospective than in prospective studies. The former are quicker, cheaper and easier to implement, but provide an inferior level of evidence compared to prospective studies. In this study, the outcome of a total of 19 315 transfused units of MB plasma (3780 transfusions) was observed. This number is considered statistically adequate to determine the safety of MB plasma.

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## Conflict of interest

The authors declare no conflict of interests.

## References

1 Lambrecht B, Mohr H, Knuver-Hopf J, *et al.*: Photoinactivation of viruses in human fresh plasma by phenothiazine dyes in combination with visible light. *Vox Sang* 1991; **60**:207–213

2 Wagner SJ: Virus inactivation in blood components by photoactive phenothiazine dyes. *Transfus Med Rev* 2002; **16**:61–66

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

4 ANSM. *Rapport d'activité hémovigilance 2011* and 2012.

5 Note for Guidance on Clinical Safety Data Management: *Definitions and Standards for Expedited Reporting*. London, European Medicines Agency, 1995:1–10

6 Proposed standard definitions for surveillance of non-infectious adverse transfusion reactions: International Society of Blood Transfusion, 2013.

7 BCSH: Guidelines for the use of fresh-frozen plasma, cryoprecipitate and cryo-supernatant. *Br J Haematol* 2004; **126**:11–28.

8 Peter C, *et al.*: Pharmacokinetics and organ distribution of intravenous and oral methylene blue. *Eur J Clin Pharmacol* 2000; **56**:247–250

9 Tinegate H, Birchall J, Gray A, *et al.*: Guideline on the investigation and management of acute transfusion reactions. Prepared by the BCSH blood transfusion task force. *Br J Haematol* 2012; **159**:143–153

10 Electronic Records; Electronic Signatures: U. S. Food and Drug Administration, 1997.

11 ICH for Good Clinical Practice E6(R1): International conference on harmonization of technical requirements for registration of pharmaceuticals for human use, 1996:1–59. www.ich.org

12 Larrea L, Castrillo A, Politis C, *et al.*: The incidence of allergic reactions with methylene blue treated plasma. A five-year European retrospective study. *Blood Transfus* 2014; **12**:s482

13 Ribon N, Narbey D, Jbilou S, *et al.*: Analyse des évènements indésirables

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receveur (EIR) allergiques graves associés à la transfusion thérapeutique de plasma sur la période 2008–2012. *Transfus Clin Biol* 2013; **20**:352

14 Bost V, Odent-Malaure H, Chavarin P, *et al.*: A regional haemovigilance retrospective study of four types of therapeutic plasma in a ten-year survey period in France. *Vox Sang* 2013; **104**:337–341

15 Politis C, Kavallierou L, Hantziara S, *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**:316–320

16 Nussbaumer W, Mayer W, Schennach H, *et al.*: Missing influence of plasma qualtiy (quarantine-stored and Methylene Blue-treated) on the frequency of side effects and the consumption of red blood cells and platelet units. *Transfusion* 2015; **55**:87A

17 Bolton-Maggs PHB (Ed), Poles D, Watt A, *et al.*: on behalf of the Serious Hazards of Transfusion (SHOT) Steering Group. The 2012 annual SHOT report, 2013.

18 Muylle L, Roisin T: *Hémovigilance en Belgique: Rapport Annuel 2012*. Brussels, Agence Fédérale des Médicaments et des Produits de Santé, 2014

19 Muylle L, Roisin T: *Hémovigilance en Belgique: Rapport Annuel 2013*. Brussels, Agence Fédérale des Médicaments et des Produits de Santé, 2015

20 Bolton-Maggs PHB (Ed), Poles D, Watt A, *et al.*: on behalf of the Serious Hazards of Transfusion (SHOT) Steering Group. The 2013 annual SHOT report, 2014.

21 European Commission: Commission Directive 2005/61/EC of 30 September 2005 implementing Directive 2002/98/EC of the European Parliament and of the Council as regards traceability requirements and notification of serious adverse reactions and events. *Off J Eur Union* 2005; **48**:32–40

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