

Letter to the Editor

Methylene blue-treated plasma: An increased allergy risk?

To the Editor:

Transfusion safety constitutes one of the major strategic goals of any transfusion system. The residual risk of transfusion-related infections has led to the development of several pathogen inactivation strategies.¹ In the second semester of 2008, in France, methylene blue (MB)-treated fresh frozen plasma (FFP-MB) replaced the usual FFP secured by quarantine (FFP-Q). This was followed by a high rate of notifications of allergic reactions to the French Health Products Agency (Agence française de sécurité sanitaire des produits de santé), which prompted a specific survey.

The rates of severe (grade 2: severe life-threatening and grade 3: resulting in death) allergic reactions to the different FFP (solvent/detergent-treated FFP [FFP-S/D], FFP-Q, and FFP-MB) reported between January 2005 and December 2009 were retrospectively compared. The strength of the relationship to the transfusion was defined by 5 causality assessment levels: (1) definite (certain), Level 4; (2) probable (likely), Level 3; (3) possible, Level 2; (4) unlikely (doubtful), Level 1; (5) excluded, Level 0.

In addition, severe allergic reactions associated with FFP-MB transfusion observed from June 2008 to December 2009 were prospectively analyzed. Briefly, plasma histamine and tryptase levels were measured at the time of severe reaction. A standardized questionnaire recording a detailed clinical history including all potentially offending agents was completed. A specific allergy workup was performed 6 weeks later. Intradermal tests with increasing concentrations of MB (pure solution 1%) starting at a 10^{-4} dilution (maximal concentration 10^{-2})² and flow cytometry basophil activation tests (Flow2 CAST, Bühlmann, France) with MB 1% and, whenever possible, with the culprit MB-treated-plasma were performed. Investigation also included skin tests

for latex and all coadministered drugs. IgA serum concentrations were systematically measured to eliminate a possible hypersensitivity reaction related to an IgA deficiency.

The frequency of severe allergic reactions to FFP-Q and FFP-S/D was similar, whereas a higher rate of reactions was observed with FFP-MB (FFP-Q: 1 in 19,269; FFP-S/D: 1 in 25,351; FFP-MB: 1 in 7,751; $P < .001$) (Table I). This difference was still observed when the comparisons were limited to reactions with imputability levels 3 and 4 ($P < .01$).

Thirty-four reactions to FFP-MB were investigated (Table II). The outcome was favorable in 31 cases; 1 death related to the allergic reactions and 2 deaths not related to transfusion were recorded. Serum tryptase concentrations were increased in 19 of the 25 cases (75%). Plasma histamine concentrations were increased in 12 of the 18 cases (66%). Skin test results were positive to MB in 3 of the 17 cases. In 2 cases, sensitization to MB was confirmed by a positive flow cytometry basophil activation test result. In 3 cases, an allergen not related to transfusion was incriminated (latex $n = 1$; protamine $n = 1$; propofol $n = 1$). In 9 cases (26%), MB responsibility was ruled out by recent previous or subsequent uneventful exposure. In 4 cases, a history of hypersensitivity reaction to a labile blood product was reported (platelet concentrate $n = 1$; FFP $n = 2$; red blood cells $n = 1$). No case of IgA deficiency was recorded.

MB for the pathogen inactivation of FFP was originally used in Germany, and FFP-MB was first produced routinely for clinical use in 1992. MB intercalates into viral nucleic acid, and subsequent illumination generates singlet oxygen radicals leading to guanosine oxidation and destruction of the viral nucleic acid, thus preventing viral replications.

Although rare, allergic reactions to MB have been reported in various clinical settings. Our results confirm that in some cases sensitization to MB could be responsible for the allergic reaction observed. However, in a larger proportion of cases, the

TABLE I. Number of severe allergic reactions (grades 2 and 3) and estimated incidence according to the imputability level (2-4, 3-4) for the different types of FFP in France between 2005 and 2009

FFP type	Year	No. of units	Imputability 2-4		Imputability 3-4	
			No. of cases	Incidence	No. of cases	Incidence
FFP-Q	2005	174,817	7	1/24,974	3	1/58,272
	2006	162,885	6	1/27,148	3	1/54,295
	2007	179,435	15	1/11,962	8	1/22,429
	2008	117,049	5	1/23,410	1	1/117,049
	2009	1,707	0		0	
	2005-2009	635,893	33	1/19,269	15	1/42,279*
FFP-S/D	2005	114,489	5	1/22,898	3	1/38,163
	2006	130,517	3	1/43,506	1	1/130,517
	2007	134,024	3	1/44,675	3	1/44,675
	2008	137,537	9	1/15,282	7	1/19,648
	2009	142,551	6	1/23,759	2	1/71,276
	2005-2009	659,118	26	1/25,351	16	1/41,195*
FFP-BM	2005					
	2006					
	2007					
	2008	58,725	11	1/5,339	5	1/11,745
	2009	204,814	23	1/8,905	9	1/22,757
	2005-2009	263,539	34	1/7,751	14	1/18,824*

*Significant differences between the different type of FFP ($P < .001$).

TABLE II. Clinical characteristics and results of allergology workup of 34 immediate hypersensitivity reactions observed during MB-treated plasma transfusion (FFP-MB)

Case no.	Sex	Age (y)	Transfusion indication	Delay to reaction (min)	Clinical symptoms	Treatment	Histamine (nmol/L)	Tryptase (g/L)	Skin prick test result	Intradermal test	BAT	Negative challenge test	Other incriminated agent
1	Female	64	Hemorrhage	10	Cardiovascular collapse Urticaria Desaturation	Epinephrine	25.8	26.7	Neg	Pos	Pos		
2	Male	35	Coagulopathy	10	Cardiovascular collapse Urticaria Dyspnea	Epinephrine	ND	ND	ND	ND	ND		
3	Male	19	Trauma	30	Cardiovascular collapse Urticaria Desaturation	Epinephrine, anti-H1	ND	4.3	Neg	Neg	ND	Pos	
4	Female	40	Plasma exchange	10	Cardiovascular collapse Dyspnea	Epinephrine, volume expansion	<0.1	17	ND	ND	ND		
5	Male	40	Trauma	15	Cardiovascular collapse Urticaria, edema Desaturation	Epinephrine	ND	ND	Neg	Neg	ND	Pos	
6	Male	77	Cardiopulmonary bypass	5	Cardiovascular collapse Urticaria	Epinephrine, norepinephrine, steroids, volume expansion	65	53	Neg	Neg	ND		
7	Male	38	Plasma exchange	5	Cardiovascular collapse, cardiac arrest Urticaria Dyspnea, desaturation	Epinephrine, cardiopulmonary bypass, steroids	ND	7.7	ND	ND	ND		
8	Female	29	Postpartum hemorrhage	10	Cardiovascular collapse Urticaria and edema Dyspnea, bronchospasm	Epinephrine, volume expansion, steroids	1	27.1	ND	ND	ND		
9	Male	29	Trauma	20	Cardiovascular collapse Urticaria	Epinephrine, steroids	28	7.37	Neg	Neg	ND		
10	Male	23	Trauma	1	Cardiovascular collapse Urticaria	Epinephrine, anti-H1, steroids	ND	ND	ND	ND	ND	Pos	
11	Female	80	Coagulopathy	10	Cardiovascular collapse Urticaria	Epinephrine	31	35.8	ND	ND	ND		
12	Male	59	Cardiopulmonary bypass	10	Cardiovascular collapse Urticaria Desaturation	Epinephrine, norepinephrine Cardiopulmonary bypass	ND	25.7	Neg	Neg	ND	Pos	
13	Male	58	Hemorrhage	15	Cardiovascular collapse Urticaria	Epinephrine	ND	ND	ND	ND	ND	Pos	
14	Male	56	Hemorrhage	5	Cardiovascular collapse Urticaria	Epinephrine, volume expansion	100	18	ND	ND	ND		
15	Male	59	Cardiopulmonary bypass	20	Cardiovascular collapse Urticaria	Epinephrine, norepinephrine, dobutamine, volume expansion	6.5	54.2	Neg	Neg	ND		

(Continued)

TABLE II. (Continued)

Case no.	Sex	Age (y)	Transfusion indication	Delay to reaction (min)	Clinical symptoms	Treatment	Histamine (nmol/L)	Tryptase (g/L)	Skin prick test result	Intradermal test	BAT	Negative challenge test	Other incriminated agent
16	Male	75	Cardiopulmonary bypass	5	Cardiovascular collapse Urticaria	Epinephrine, anti-H1	ND	ND	Pos	ND	Pos		
17	Male	48	Trauma	10	Cardiovascular collapse Dyspnea	Epinephrine	82	65.8	ND	ND	ND		
18	Female	63	Nephrectomy	5	Cardiovascular collapse Urticaria Dyspnea	Steroids, anti-H1	187	33.2	ND	ND	ND	Pos	
19	Male	16	Trauma	15	Cardiovascular collapse Erythema Bronchospasm	Epinephrine	28.4	7.7	Neg	Neg	ND	Pos	Propofol
20	Male	26	Trauma	5	Hypotension Urticaria, angioedema Bronchospasm	Epinephrine, anti-H1, steroids	ND	ND	Neg	Neg	ND		
21	Female	30	Postpartum hemorrhage	15	Cardiovascular collapse Urticaria, pruritus, and edema Dyspnea	Ephedrine, salbutamol, anti-H1, steroids	ND	13.7	Neg	Neg	ND		
22	Male	17	Trauma	20	Hypotension Bronchospasm	Epinephrine, salbutamol, steroids	ND	5	Neg	Neg	Neg	Pos	
23	Male	63	Cardiopulmonary bypass	10	Cardiovascular collapse Urticaria	Steroids	ND	ND	ND	ND	ND		
24	Male	85	Cardiopulmonary bypass	10	Cardiovascular collapse	Epinephrine	5.4	10.2	Neg	Neg	Neg		Protamine
25	Female	33	Postpartum hemorrhage	1	Hypotension Pruritus, angioedema Dyspnea		<1	62.7	ND	ND	ND		
26	Female	84	Orthopedic	25	Cardiovascular collapse Erythema Desaturation	Volume expansion, ephedrine, norepinephrine	537	62	Neg	Neg	Neg		
27	Male	79	Vascular surgery	35	Cardiovascular collapse	Ephedrine, norepinephrine, volume expansion	ND	61.2	ND	ND	ND	Pos	
28	Female	11	Trauma	30	Cardiovascular collapse Urticaria Desaturation	Norepinephrine, volume expansion	100	13.8	ND	ND	ND		
29	Female	80	Cardiopulmonary bypass	2	Cardiovascular collapse, cardiac arrest	Dobutamine	ND	29.8	ND	ND	ND		
30	Male	7	Plasma exchange	5	Cardiovascular collapse Urticaria, edema Dyspnea	Epinephrine, steroids	94	17.2	ND	ND	Neg		
31	Female	47	Plasma exchange	60	Hypotension Urticaria, angioedema Dyspnea	Steroids	ND	ND	Neg	Neg	ND		
32	Female	30	Postpartum hemorrhage	5	Cardiovascular collapse Urticaria, angioedema	Neosinephrine, volume expansion, steroids	ND	10	Neg	Neg	ND		Latex

(Continued)

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Case no.	Sex	Age (y)	Transfusion indication	Delay to reaction (min)	Clinical symptoms	Treatment	Histamine (nmol/L)	Tryptase (g/L)	Skin prick test result	Intradermal test	Negative challenge test	Other incriminated agent
33	Female	18	Trauma	30	Cardiovascular collapse Urticaria Bronchospasm	Volume expansion, epinephrine, corticoid, salbutamol	21.8	19.3	Neg	Pos	ND	
34	Male	32	Coagulopathy		Cardiovascular collapse Urticaria Dyspnea, desaturation	Epinephrine, volume expansion	ND	ND	ND	ND	ND	

BAT, Basophil activation test; ND, not done; Neg, negative; Pos, positive.

responsibility of MB was ruled out. This supports the hypothesis of an increased allergic risk related to the pathogen inactivation process itself.

Since its introduction, the process has been significantly modified. In France (Theraflex MB-Plasma system; MacoPharma, Tourcoing, France), it is carried out on individual units of plasma in 8 blood banks. A 0.65- μ m membrane filter (Plasmaflex PLAS4; MacoPharma) is used to remove residual leucocytes, red blood cells, platelets, and aggregates. The filtered plasma then flows on a dry pill of 85 μ g anhydrous MB chloride integrated in the bag system. The illumination is performed at a temperature maintained at $\leq 22^{\circ}\text{C}$ at a peak wavelength of 590 nm during 20 minutes to achieve a light dose of 180 J/cm². After treatment, more than 90% of the residual MB combined with its photo products is removed by a specially designed filter to an average remaining concentration of 2 μ g/L (Blueflex; MacoPharma).

The effect of pathogen reduction by MB and illumination on the integrity of plasma proteins has been addressed by several studies reporting significant alterations.^{3,4} In contrast, the standardized S/D inactivation method was, apparently, not associated with significant modification of the gel-electrophoretic profile of plasma proteins.⁵ Leukofiltration as well as MB filtration might also have played a role in the pathogenesis of the increased risk of allergic reaction we report.⁶ Our results support the need to promote extensive proteomic analysis studies to further characterize protein modifications and/or otherwise unexpected neoantigen production that could be involved in the genesis of the hypersensitivity reactions we observed.

Our report represents the first series suggesting a possible increased risk of hypersensitivity reactions induced by the use of FFP-MB on a large scale. No increase in the number of hypersensitivity reactions related to the use of FFP-MB has been previously reported in other countries.⁷ However, differences in the rate of reaction resulting from qualitative differences between plasma produced in several countries have already been reported.⁸ Such differences may depend on each process of FFP-MB production used in these countries. No filtration step is used in Germany, whereas guidelines recommend an MB concentration below 30 mg per unit in the United Kingdom and below 30 mg/L in France. FFP-MB is produced with apheresis plasma donation in France and with whole blood plasma donation in many other countries. Differences regarding the efficacy of the adverse effects reporting system in countries using FFP-MB might also have played a role. Finally, a possible bias concerning the rate of declaration could be related to the clinician awareness of a

possible increased rate of allergic reactions with FFP-MB following the prospective survey decided by Agence française de sécurité sanitaire des produits de santé. However, one should notice that this increased rate of allergy reports has been rapidly detected before the active survey.

Although the mechanism of this increased risk of allergic reaction remains to be elucidated, our results are a strong incentive for a thorough reevaluation of the benefit-to-risk ratio of pathogen reduction using MB.

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