

Anaphylaxis to Gelofusine[®] confirmed by *in vitro* basophil activation test: a case series[★]

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Summary

The plasma expander Gelofusine[®] (succinylated gelatin) is a recognised cause of peri-operative anaphylaxis. Current diagnosis of Gelofusine sensitivity is by skin testing, a procedure that itself carries a risk of allergic reaction. We evaluated the reliability of the *in vitro* basophil activation test as a diagnostic assay for Gelofusine sensitivity in subjects with a clinical history highly suggestive of Gelofusine allergy. Six patients with peri-operative anaphylaxis clinically attributed to Gelofusine were skin tested to confirm sensitivity. Control subjects included three healthy subjects and five subjects allergic to a neuromuscular blocking drug, all negative on Gelofusine skin testing. Whole blood basophil activation to Gelofusine was analysed by flow cytometry for CD63 surface expression. All of the Gelofusine sensitive patients and one of the control allergic subjects showed positive basophil activation to Gelofusine. In this series of subjects, the basophil activation test for Gelofusine allergy had a sensitivity of 100% and a specificity of 87.5%. Our findings suggest that basophil activation testing is a safe and reliable *in vitro* assay for prediction or confirmation of Gelofusine sensitivity in patients with high clinical suspicion of Gelofusine-induced anaphylaxis.

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Anaphylaxis due to sensitivity to Gelofusine[®] (succinylated gelatin) and other plasma expanders is well described, usually causing reactions during resuscitation or anaesthesia [1–4] with an estimated incidence of 0.07–0.15% [5]. Allergy to gelatin has been associated with anaphylactic reactions to vaccination, with one quarter of children suffering from anaphylaxis to the Measles-Mumps-Rubella vaccine demonstrating IgE antibody production to gelatin [6, 7]. These individuals may be at risk of reaction to other gelatin-containing products including Haemaccel[®] and Gelofusine [4,8].

The mechanism of allergic reactions to Gelofusine is unclear, with both IgE and non-IgE mechanisms being proposed [3]. To date, the diagnosis of sensitivity to Gelofusine relies on skin prick and/or intradermal testing for confirmation, a personnel-intensive and

time-consuming process [4] which itself carries a risk of anaphylaxis. A reliable blood test to predict or confirm allergy to Gelofusine would enable a simpler and safer management strategy for individuals with possible Gelofusine allergy. The flow cytometric basophil activation test (BAT), with detection of surface expression of CD63 (a lysosomal membrane glycoprotein) on activated basophils, has been useful in identifying subjects allergic to neuromuscular blocking drugs [9] and beta-lactams [10] and more recently we have used this assay to confirm hypoallergenic latex allergens [11]. We report a case series where peri-operative anaphylaxis due to Gelofusine was confirmed by skin testing and BAT, suggesting that the BAT is a reliable screening test for Gelofusine allergy in such patients, avoiding the risks associated with skin testing.

Methods

This study was approved by The Alfred Hospital Ethics and Research Committee and signed informed consent for the procedures and publication of data ensuring confidentiality was obtained from all subjects. Six patients with peri-operative anaphylaxis (clinically attributed to Gelofusine) were recruited from the Drug Allergy Clinic at the Alfred Hospital, Melbourne, Australia. Three healthy control subjects and five control patients with confirmed sensitivity to a neuromuscular blocking drug (two sensitive to succinylcholine, two sensitive to atracurium and one sensitive to rocuronium) were also studied.

All subjects underwent skin testing with Gelofusine (neat). Skin prick and intradermal testing (0.02 ml) were performed as described previously [3]. A positive skin prick test was defined by a wheal diameter >3 mm at 15 min and a positive intradermal skin test was defined by a flare reaction >15 mm and/or a wheal greater than the saline control at 20 min.

In vitro challenge of basophils with Gelofusine

All participants underwent the BAT with Gelofusine, employing a technique using whole blood samples modified from that described by Sanz *et al.* [12]. Heparinised blood (100 µl) was incubated with 20 µl stimulation buffer containing 2 ng.ml⁻¹ IL-3 (R & D Systems, Minneapolis, MN) and 5000 IU heparin in 0.2 ml (David Bull Laboratories, Victoria, Australia) for 10 min at 37 °C. Gelofusine 100 µl (B. Braun, Castle Hill, NSW, Australia), diluted 1 : 5 and 1 : 2 in stimulation buffer and neat (equivalent to 3.6, 9 and 18 mg.ml⁻¹ succinylated gelatin, respectively), or gelatin (10 and 40 µg.ml⁻¹ in

stimulation buffer), was added and the cells further incubated for 20 min at 37 °C. Positive control cell samples were stimulated with rabbit antihuman IgE antibody (7.5 µg.ml⁻¹ in stimulation buffer; DAKO Corporation, Carpinteria, CA) or fMLP (0.4 µg.ml⁻¹ in stimulation buffer; Sigma, St. Louis, MO). Negative controls included cells incubated with stimulation buffer alone. Cells were then stained with PE-conjugated mouse antihuman CD63 (Caltag Laboratories, Burlingame, CA) and FITC-conjugated goat antihuman IgE (Caltag Laboratories). Red blood cells were lysed and remaining cells pelleted by centrifugation, washed and resuspended in 100 µl wash buffer per tube. 7-Amino-actinomycin D (7AAD; 2.5 µg.ml⁻¹; Sigma) was added to each tube and flow cytometric analysis performed using a FACScalibur flow cytometer and CELL QUEST software (BD Biosciences, San Jose, CA).

Approximately 300 000 total events were collected per test to obtain sufficient basophils for reliable analysis. Cells were first gated on a forward scatter *vs* side scatter region, later validated to contain basophils by back-gating of high IgE (IgE^{hi}) staining cells. The expression of CD63 on viable (7AAD negative) IgE^{hi} cells was then determined (Fig. 1). Both B cells and monocytes, which may stain weakly for IgE, were shown to be excluded from the gated IgE^{hi} cell population by CD19 and CD14 staining, respectively (data not shown). The threshold for positive CD63 staining was based on the discrimination between the negative control (no antigen control) and positive control staining (fMLP and anti-IgE stimulation; Fig. 1b) as well as isotype control staining. The percentage of basophil activation for test samples was corrected by subtracting spontaneous CD63 expression (negative control). A receiver operator characteristic (ROC) curve

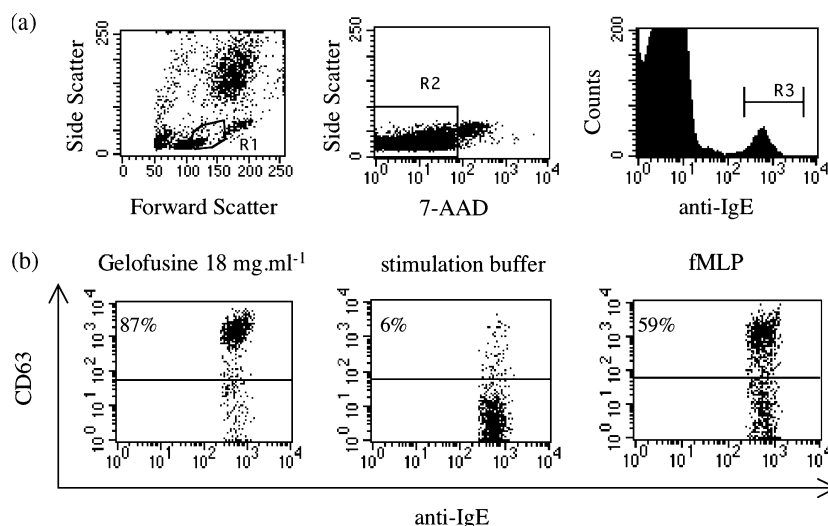


Figure 1 Flow cytometric analysis of activation of blood basophils from a Gelofusine sensitive patient by Gelofusine. a) Viable basophils are identified by forward and side scatter (R1), exclusion of the vital dye 7AAD (R2) and high anti-IgE staining (R3). b) CD63 expression on gated basophils incubated with Gelofusine (18 mg.ml⁻¹), no added allergen (i.e. stimulation buffer alone, negative control) and fMLP (positive control).

Subject No.	Age; years	Sex	Clinical reaction to Gelofusine	Intradermal skin test	
				Wheal; mm	Flare; mm
1	57	F	Severe hypotension	10	34
2	71	M	Severe hypotension, rash	3	11 × 10
3	65	M	Severe hypotension	2	20 × 25
4	67	M	Severe hypotension, rash, arrhythmia	4	40
5	80	M	Severe hypotension	20 × 18	25 × 25
6	85	F	Severe hypotension, arrhythmia	12	30

Table 1 Characteristics of patients sensitive to Gelofusine.

analysis (SPSS 12.0.1) was used to determine the best cut-off with regard to the highest sensitivity and specificity.

Results

The six patients who had peri-operative anaphylaxis clinically attributable to Gelofusine had a positive skin test to Gelofusine. Patient characteristics, features of allergic reactions and skin testing results for each Gelofusine-allergic subject are given in Table 1. The mean (SD) size of intradermal skin-test flares in the positive patients was 25.2 (11.8) mm. The five control patients who had peri-operative anaphylaxis due to neuromuscular blocking drugs (two male and three female; aged 14–72 years) and three healthy controls (one male and two female, aged 31–50 years) had negative skin tests to Gelofusine. Evaluation of BAT results at different concentrations of Gelofusine for all subjects by ROC analysis showed that a threshold of 3.6% for Gelofusine added neat (18 mg.ml^{-1} succinylated gelatin) gave an optimal sensitivity of 100% and a specificity of 87.5% (Fig. 2). At this

concentration of Gelofusine, the BAT was positive for the six confirmed Gelofusine sensitive patients and negative for all the control subjects except one of the neuromuscular blocking drug allergic patients (subject No. 13; Fig. 3).

Testing of patients' blood basophil activation to Gelofusine diluted 1 : 2 and 1 : 5 showed a lower percent basophil activation and a decrease in sensitivity, except for some of the control patients allergic to neuromuscular blocking drugs (Fig. 3). None of the subjects had a history of allergy to gelatin; however, one patient from the Gelofusine sensitive group (subject No. 2) tested positive to gelatin at a concentration of 40 µg.ml^{-1} but not 10 µg.ml^{-1} and subject 13 of the neuromuscular blocking drug allergic group tested positive to gelatin at a concentration of 40 µg.ml^{-1} (data not shown). All subjects tested positive to the control fMLP and all but one tested positive to antihuman IgE antibody (data not shown).

Discussion

This study demonstrates the potential utility of the BAT for diagnosis of Gelofusine sensitivity in subjects with a clinical history highly suggestive of Gelofusine allergy. To our knowledge, this is the first study to describe the use of the BAT in this context. An algorithm for investigation of subjects with suspected Gelofusine allergy based on our results is presented in Fig. 4.

The basophil activation assay has previously been used to diagnose drug allergy, particularly for sensitivity to beta-lactams, where test sensitivity approaches 50% [10]. For allergy to non-steroidal anti-inflammatory agents, the BAT has been shown to have a sensitivity of 63% and a specificity of 93% [13] and for anaesthetic allergy due to neuromuscular blocking drugs the sensitivity of the BAT is 79% [14]. The high sensitivity and specificity of this test in our study supports its potential role in the investigation of allergy to Gelofusine. Peak response occurred at the highest concentration of Gelofusine in all patients with Gelofusine allergy confirmed by skin test. For routine diagnostic screening we recommend the addition of undiluted Gelofusine to blood samples.

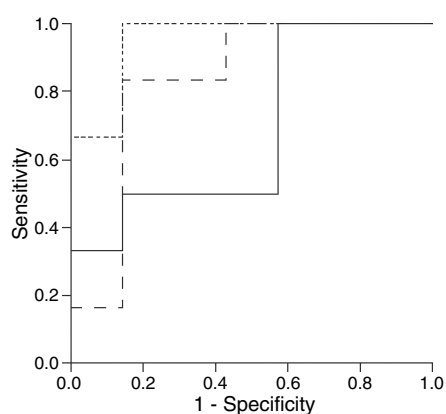


Figure 2 Receiver operating characteristic curve for CD63 expression on basophils incubated with Gelofusine diluted 1 : 5 (—), 1 : 2 (---) in stimulation buffer and neat (.....) from all subjects demonstrating the determination of the positive threshold for Gelofusine sensitivity. A threshold of 3.6% for Gelofusine added neat (18 mg ml^{-1} succinylated gelatin) gave optimal sensitivity (100%) and specificity (87.5%).

Figure 3 Summary of CD63 expression on basophils incubated with Gelofusine diluted 1 : 5 (solid bar) or 1 : 2 (white bar) in stimulation buffer and neat (grey bar) for patients sensitive to Gelofusine (1–6), healthy controls (7–9) and control patients allergic to neuromuscular blocking drugs (10–14). The dotted line shows the cut-off of 3.6% as determined by a receiver operating characteristic curve analysis.

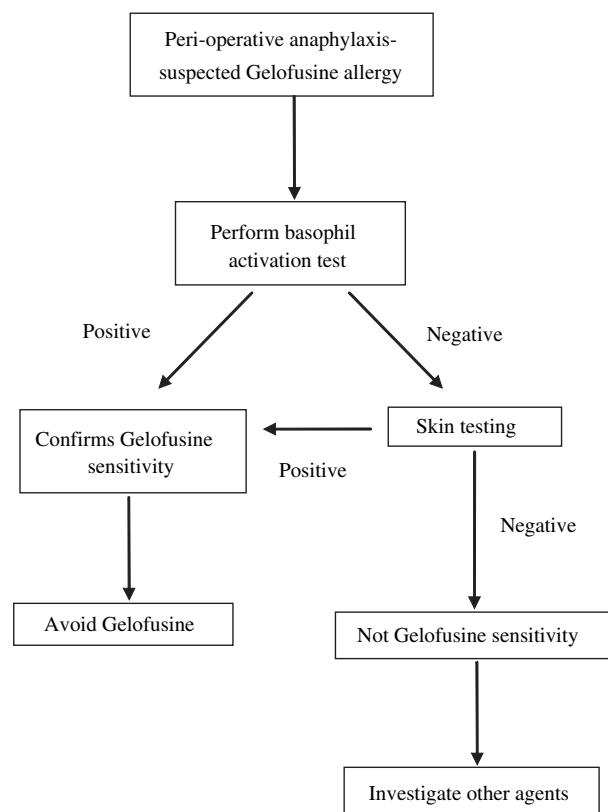
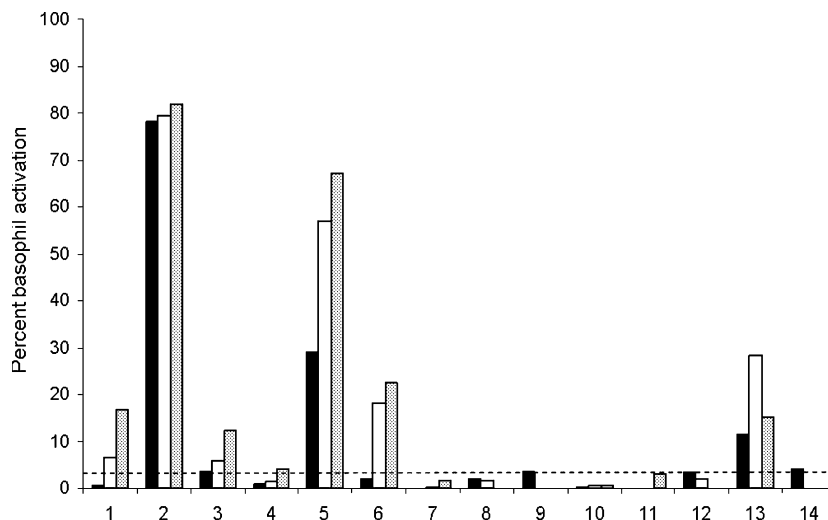


Figure 4 Algorithm for investigation of suspected sensitivity to Gelofusine.

Whilst in our study the negative predictive value of BAT for Gelofusine is high (100%), our group was carefully selected with a high pretest probability of allergy to Gelofusine. Study of a larger population would be needed to ascertain the utility of the BAT as a screening test where pretest probability is low.

On the basis of this study, the BAT is as reliable as skin testing in the diagnosis of Gelofusine allergy when the clinical suspicion is high and provides a convenient and safe way of confirming likely diagnoses in situations where skin testing is not readily available or desirable.

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