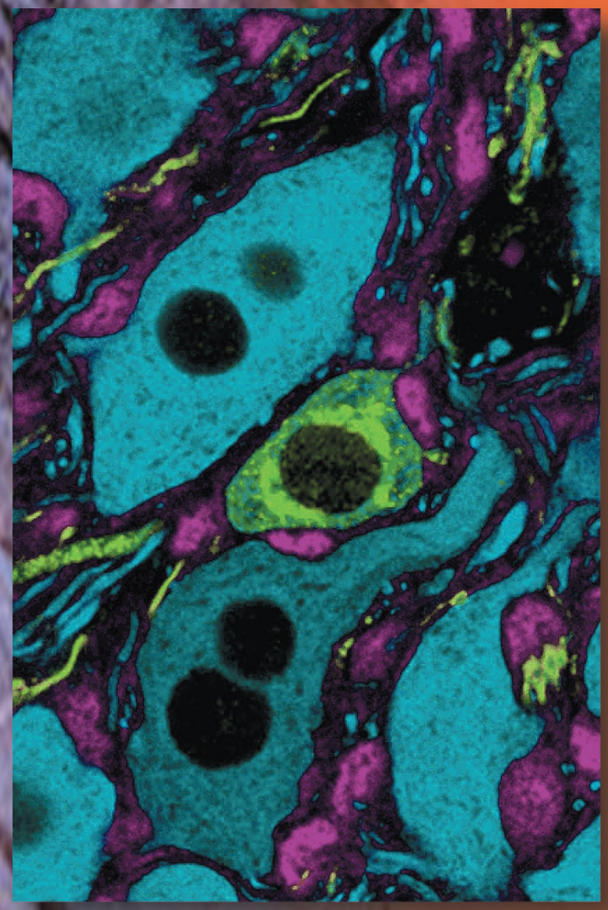
FEBRUARY 2021 VOL. 42 No. 1 Pages 1 - 42 AUSTRALIAN JOURNAL OF MEDICAL SCIENCE

AUSTRALIAN JOURNAL OF
Medical Science
2021



February 2021
Vol. 42
No. 1

ORIGINAL ARTICLES

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Australian Journal of Medical Science

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Chief Executive: Mr Michael Nolan, BAppSc BA MPhil GradCertMgt FAIMS MASM

Design, formatting and administration: Ms Simona Adochiei
Email: programs@aims.org.au
Website: www.aims.org.au
Telephone 61 7 3876 2988
Address: PO Box 1911 Milton Qld 4064 Australia

The Australian Journal of Medical Science is the official publication of the Australian Institute of Medical and Clinical Scientists (AIMS).

Circulation 2000 per issue. The Journal is circulated to members in pathology laboratories, universities and research institutes throughout Australia and overseas.

Annual subscription rates are available from AIMS National Office.

Article reprints may be organised on request from AIMS National Office.

Advertising rates are available from AIMS National Office.

Abstraction of the Australian Journal of Medical Science is through the following serial catalogue listings: Australasian Medical Index, Chemical Abstracts, and EMBASE/Excerpta Medica.

The Australian Journal of Medical Science is included on the Australian Research Council ERA 2018 Journal List.

ISSN 1038-1643

Printed by Westminster Eagle Eye Printing, PO Box 161, Paddington Qld 4064.

Design Cover and layout design by Kim Brown, 23 Denman St Exeter SA 5019 kimbo@internode.on.net. Cover photograph courtesy of Prof Ian Gibbins, Flinders Medical Centre, Adelaide.

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A diagnostic menACE: the role of ACE-inhibitors in acute isolated hypotensive transfusion reactions and examination of the utility of tryptase levels

Kylie Fitch[1], Rebecca Adams[1]

[1]Sullivan Nicolaides Pathology, Bowen Hills Laboratory, Brisbane, Queensland

Abstract

Isolated acute hypotensive transfusion reactions (AHyTR) are considered a rare event, so much so that they are not included in all acute transfusion reaction investigation protocols or guides. The majority of AHyTR reported to be linked to a transfusion relate to the concurrent use of angiotensin-converting enzyme (ACE) inhibitor medication. If the correlation between ACE inhibitor (ACEi) and hypotensive reaction is not recognised there is potential for unnecessary testing to elucidate the cause, and delays in providing patients the blood products they require. We describe the investigation and transfusion management of a 68-year-old male patient who suffered two consecutive AHyTRs, which were later determined to be most likely due to the concurrent use of ACEi. We also review the differential diagnosis of AHyTR, involvement of bradykinins in the pathogenesis and then examine the diagnostic value of tryptase levels post transfusion reaction.

Keywords: *Acute hypotensive transfusion reaction, angiotensin-converting enzyme inhibitor, transfusion reaction investigation protocols, bradykinins, tryptase*

Introduction

Acute transfusion reactions are those occurring <24h after transfusion and are commonly divided into immunological and non-immunological categories (Sanders *et al* 2007; Savage and Hod 2017). The clinical symptom of hypotension following transfusion can be associated with both immunological and non-immunological causes. Hypotension is commonly associated with other symptoms including fever, dyspnoea, urticaria or rash as part of inflammatory or allergic reactions in severe febrile non-haemolytic transfusion reactions (FNHTR), transfusion-transmitted bacterial infection (TTBI), acute haemolytic transfusion reactions (AHTR), transfusion-associated acute lung injury (TRALI), or anaphylaxis (Sanders *et al* 2007; Savage and Hod 2017).

Only a small number of cases of isolated severe hypotension in the absence of other signs of inflammation, allergy, or haemorrhage have been previously documented. This lack of reported cases may be the reason the scenario of isolated severe AHyTRs is not always included in transfusion reaction investigation protocols or guides, and not always considered in the differential diagnosis by laboratory or clinical staff.

Address correspondence to:
Kylie Fitch
Sullivan Nicolaides Pathology
24 Hurworth Street, Bowen Hills, 4006
Queensland
E mail: kylie_fitch@snp.com.au

The diagnosis of AHyTR is one of exclusion, and there are no specific confirmatory assays readily available outside research laboratories. The identification of symptoms and immediately responding to transfusion reactions is the responsibility of clinical staff, however the subsequent investigation of these reactions becomes the combined responsibility of the treating clinician, the pathology laboratory and the blood service. Determining the underlying cause of the reaction in a timely manner is critical for immediate intervention and is essential for future transfusion management of the patient. We describe a case of a rare AHyTR with the aim to increase familiarity for medical professionals confronted with this unusual presentation. The utility of tryptase levels in these patients is then examined to determine the causes of tryptase elevation and the link between tryptase elevation and ACE inhibition.

Method

Case study and literature review. Bibliographies of the included studies or articles were also searched for additional references.

Case presentation

A 68-year-old male developed hypotension with a significant drop in blood pressure (BP) from 130/60 to 51/33 in conjunction with flushing, feeling hot, throat tightening and visual disturbance within 30 seconds of commencement of transfusion of a group B RhD Positive (BPOS) pooled platelet unit for severe thrombocytopenia. He has a past history of haemochromatosis requiring venesection, metastatic colorectal adenocarcinoma, cholangiocarcinoma, liver resection and therapy-related acute myeloid leukaemia (t-AML) for which he was receiving treatment. He was blood group BPOS with no red cell antibodies and had previously been transfused blood products during his t-AML induction therapy with no transfusion reactions recorded. The patients BP corrected to 130/60 along with rapid resolution of all other symptoms following administration of 100mg hydrocortisone, 10mg loratadine, and 1g paracetamol (patient observations are detailed in Table 1). Our laboratory was informed of the reaction after resolution of the event. There was no evidence of haemolysis following the event, the patient remained afebrile, and urinalysis was normal. Given severe thrombocytopenia, with a platelet count of 13 x 10^9/L demonstrated on post-transfusion full blood count (FBC) and no platelet increment due to the very small volume infused, a further unit was prescribed the following day by the treating haematologist. Premedication of loratadine and intravenous hydrocortisone was prescribed for this transfusion. After 1 min and infusion of only approximately 5mL of the subsequent group B RhD Negative (BNEG) pooled platelet unit the patient developed symptoms of flushed face and throat irritation with the BP reading at this time unable to be obtained as it was below measurable range of the device. The transfusion was immediately ceased and rapid resolution of symptoms and BP recovery within the next 10-15 min was documented without the need for further medications. Urine visual check for haemolysis was clear, FBC and electrolytes and liver function tests (ELFT) did not reflect a haemolytic picture, and the patient was again afebrile with no skin rash, oedema or respiratory distress.

Both events were reviewed by a multidisciplinary team including laboratory haematologists, a senior laboratory blood bank scientist and a blood service medical officer. It was at this point that the isolated hypotensive reaction was suggested to possibly be related to ACEi therapy. Further enquiries with the treating clinician did reveal that the patient was in fact on ACEi with a recent dose increase preceding the first reaction. ACEi treatment was suspended for approximately 48 h prior to a further transfusion which was uneventful. The rarity of AHyTR prompted an attempt to exclude an atypical presentation of an allergic reaction with retrospective addition of tryptase testing. Tryptase levels on the samples collected at the time of both the first and second reaction were elevated at 20.6 µg/L and 19.2 µg/L (RR <15 µg/L) respectively (Figure 1). The tryptase level three days after the reactions and following the uneventful transfusion returned to within the reference range (12.9µg/L). This tryptase level was also obtained three days after stopping treatment with the ACEi. A viable frozen serum sample was analysed from before the patient had ever received blood products, had their ACEi dose increased and before either of the transfusion reactions gave an elevated tryptase level of 51.1 µg/L (Figure 2). It was postulated that this elevation was due to the patient undergoing induction chemotherapy for t-AML at the time.

The patient went on to successfully receive further blood products with hydrocortisone and loratadine premedication before again suffering reactions following red cell and platelet transfusion over two consecutive days. There was no hypotension and the symptoms were reported by the patient to include jaw pain and general distress about continuing, however they resolved quickly after transfusion was ceased (Table 1). Due to the history of reactions with no clear cause and now with red cell units also involved, a full blood bank serological workup was performed including assessment of Immunoglobulin A (IgA) levels and anti-IgA antibodies. Laboratory investigation for red cell incompatible transfusion reaction excluded the presence of a haemolytic transfusion reaction and several sets of blood cultures remained sterile after five days. The anti-IgA testing excluded the presence of IgA antibody-related anaphylaxis. Human platelet antigen (HPA) and human leukocyte antigen (HLA) testing at the request of the treating clinician were performed to investigate the cause of the platelet refractoriness independent of the transfusion reactions. The HLA antibody testing did not confirm or exclude a possible inflammatory reaction due to HLA antibodies as multispecific HLA Class 1 antibodies were detected with a frequency of 13% panel reactive antibody (cPRA-calculated PRA) (Soumya 2012). The report however excluded platelet HPA incompatibility and did not recommend that this patient requires HLA matched platelets. Potential for anxiety causing the latest reactions was considered highly likely by the treating clinician and the patient was offered transfer to the Intensive Care Unit (ICU) and mild sedation prior to the next transfusion. This transfusion was uneventful as was all subsequent transfusions. Table 2 details all products provided during this period with Table 3 summarising laboratory results obtained over the investigation time frame.

Discussion

Part of the diagnostic challenge for assessment of transfusion reactions relates to the fact that many clinical

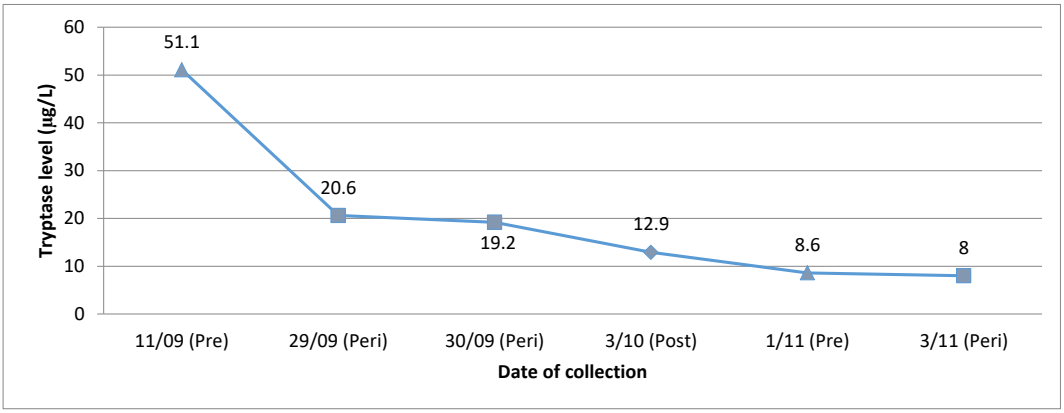

Figure 1. *Tryptase levels pre, peri and post transfusion reactions*

signs and symptoms are associated with more than one type of reaction, as is the case with hypotension. Table 4 shows the various transfusion reactions known to be associated with hypotension divided into immunological and non-immunological classifications.

Acute or immediate transfusion reactions occur within 24 h of the transfusion and often during the transfusion (Savage and Hod 2017). In this patient, several possible causes of hypotension were excluded, however acute isolated hypotension due to ACEi was not initially considered, due to the rarity and limited awareness of this aetiology.

Causes of acute hypotension within minutes of commencing transfusion are most commonly as a result of anaphylaxis, acute haemolysis or acute hypotensive reactions, with TRALI occurring within 6 h of transfusion (usually within 1-2 h), and bacterial sepsis occurring during or up to 4-8 h after transfusion (Hume and Robillard 2012). A brief review of causes of acute onset hypotension as a component of more common transfusion reactions follows, as well as a discussion of how these relate to our patient's presentation and laboratory investigations.

Acute haemolytic reactions

AHTR are preventable in most cases but if they occur they are difficult to treat with a high mortalitiy rate. They are characterised by accelerated destruction of incompatible red cells, and are more commonly associated with the transfusion of red cells, however may also occur with the transfusion of plasma containing products (e.g. fresh frozen plasma or platelets) that contain a high titre of antibodies directed towards the recipient's red cell antigens. The clinical presentation of AHTR can vary in severity, however fever and/or chills and acute shortness of breath remain the most common initial symptoms. Table 4 shows other symptoms seen in AHTR. Hypotensive shock associated with severe AHTR occurs with intravascular haemolysis (rarely with extravascular haemolysis). Complement activation by alloantibodies is the likely causal factor, with complement components C5a and C3a considered anaphylatoxins with potent proinflammatory and vasodilatory effects. Similarly, activation of Factor XII (FXII) leads to bradykinin (BK) release and this will be discussed later. In addition, immune-mediated haemolysis stimulates the production of cytokines tumor necrosis factor alpha (TNF-α) and interleukin 1 (IL-1) which can cause hypotension by promoting nitric oxide production from endothelial cells (Robertson and Davenport 2012). Other complications can include acute kidney injury and disseminated intravascular coagulation (DIC) with microvascular bleeding. The severity of the reaction is related to the amount of incompatible blood product transfused, with high titres (>1000) of anti-A or anti-B implicated in low-volume (<200mL) transfusion reactions (Berséus *et al* 2013).

Laboratory investigations to detect intravascular haemolysis include urinalysis or visual check for haemoglobinaemia and haemoglobinuria, FBC +/- film and reticulocyte count, visual check of patient and donor unit plasma for haemolysis, haptoglobin, lactate dehydrogenase (LDH), bilirubin, and renal function checks (electrolytes and creatinine). If DIC is suspected, a coagulation profile including prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen and D-Dimer are performed. It is useful to compare results to a pre-transfusion sample where possible and be alert to the fact that some clinical conditions can mask the presence of new haemolysis. To determine whether haemolysis has an immunological cause, the serological workup should involve repeating the pre-transfusion testing and verifying the accuracy of all

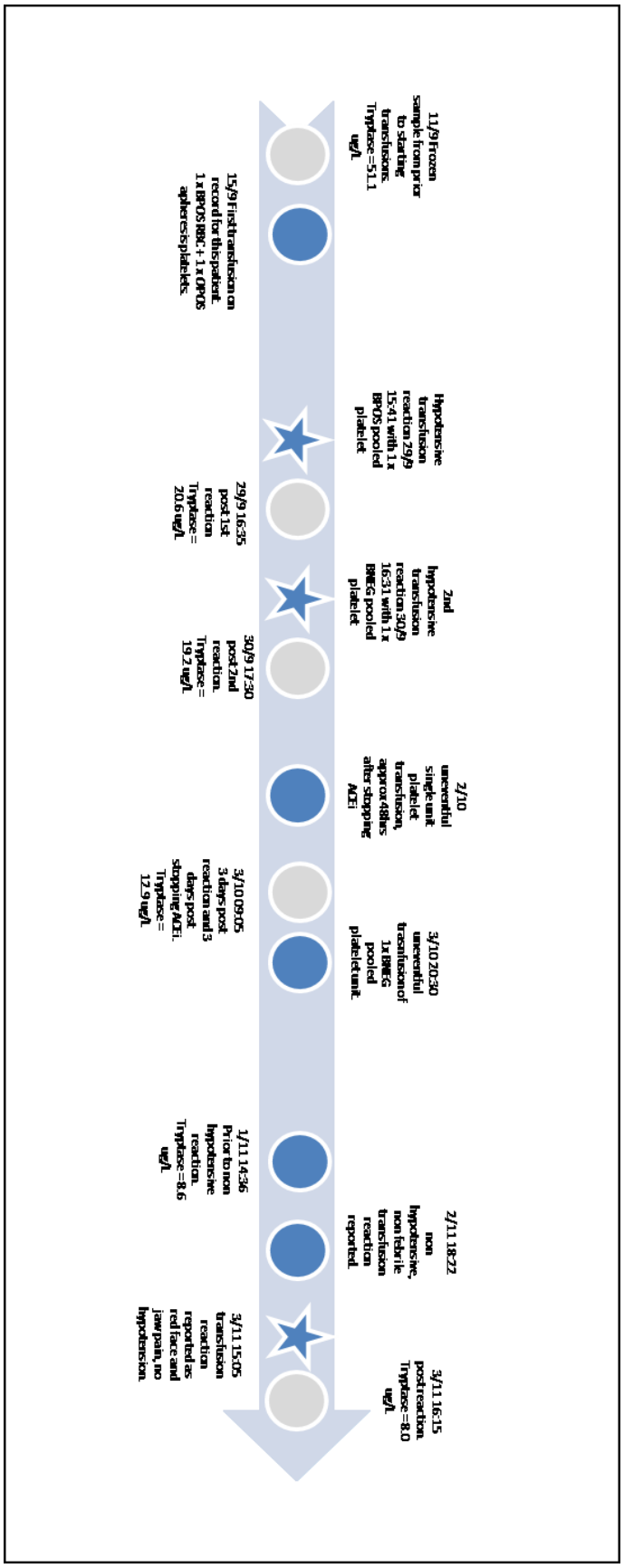

Figure 2. *Timeline showing tryptase testing and reactions.*

Table 1. *Clinical symptoms and observations during transfusion reactions*

1st reaction - BPOS pooled platelet unit. No premedications.		**Date/time (pre-transfusion): 29/9 15:40**	**Date/time (post-transfusion): 29/9 15:41**	**Date/time (post-transfusion): 29/9 16:00**	**Date/time (post-transfusion): 29/9 16:05**
Date/ time transfusion commenced	29/9/19 15:40				
Approx volume transfused (ml)	10-20ml				
Time symptoms observed	15:41				
Temperature		36.4	36.5	36.4	36.5
Blood pressure		150/60 *	185/65	51/33	130/60
Pulse		68	92	60	69
Chills/rigors (Y/N)		N	N	N	N
Skin rash (Y/N)		N	N	N	N
Dyspnoea/ SpO2		96% RA	89% RA	96% 2L NP	100% 2L NP
Other signs or symptoms		Red face, felt hot, tightening of throat, visual disturbances			Symptoms resolved

* BP not taken immediately before starting the transfusion as hospital policy is to avoid in thrombocytopenic patients. This BP was taken 29/9 at 05:00.

2nd reaction- BNEG pooled platelet. Pre-medication- hydrocortisone IV, loratadine PO.		**Date/time (post-transfusion): 30/9 16:32**	**Date/time (post-transfusion): 30/9 16:35**	**Date/time (post-transfusion): 30/9 16:42**	**Date/time (post-transfusion): 30/9 16:55**
Date/ time transfusion commenced	30/9/19 16:30				
Approx volume transfused (ml)	5 ml				
Time symptoms observed	16:31				
Temperature		–	–	–	–
Blood pressure		203/89	160/55	*	189/75
Pulse		100	85	69	70
Chills/rigors (Y/N)		N	N	N	N
Skin rash (Y/N)		N	N	N	N
Dyspnoea/ SpO2		96%	98%	99%	98%
Other signs or symptoms		Flushed face,slight throat irritation.			Symptoms resolved

Note: Temperature measurements were not provided by hospital. Verbal details stated non-febrile. No pre-transfusion measurements provided for this event.

* BP unable to be obtained as ward stated below device readable range.

3rd reaction- BPOS pooled platelet.-remedication-100mg hydrocortisone IV,10mg loratadine PO. Single unit PRBC given 2 h earlier.		**Date/time (post-transfusion): 2/11 18:20**	**Date/time (post-transfusion): 2/11 18:24**	**Date/time (post-transfusion): 2/11 18:30**	**Date/time (post-transfusion): 2/11 18:40**
Date/ time transfusion commenced	2/11/19 18:20				
Approx volume transfused (ml)	5-10 ml				
Time symptoms observed	18:22				
Temperature		36.1	36.3	36.1	37.2
Blood pressure		130/80*	125/60	103/63	104/66
Pulse		85	80	92	84
Chills/rigors (Y/N)		N	N	N	Y
Skin rash (Y/N)		N	N	N	N
Dyspnoea/ SpO2		98% RA	84% for 30sec-> 98% on 15L O2	100%	100
Other signs or symptoms		Syncope, GCS= 1			Further dose : 100mg hydrocortisone IV, 10mg loratadine PO.

* BP not taken immediately before starting the transfusion as hospital policy is to avoid in thrombocytopenic patients. This BP taken 2/11 at 16:10.

4th reaction- BPOS pooled platelet.-Premedication-100mg hydrocortisone IV,10mg loratadine PO. 1hr prior to transfusion.		Date/time (post-transfusion): 3/11 15:00	Date/time (post-transfusion): 3/11 15:05	Date/time (post-transfusion): 3/11 15:10	–
Date/ time transfusion commenced	3/11/19 15:00				
Approx volume transfused (ml)	50 ml				
Time symptoms observed	15:05				
Temperature		36.5	36.5	36.5	–
Blood pressure		135/70	150/95	125/65	–
Pulse		80	90	85	–
Chills/rigors (Y/N)		N	N	N	–
Skin rash (Y/N)		N	N	N	–
Dyspnoea/ SpO2		>95% RA	>95% RA	>95% RA	–
Other signs or symptoms		Face red, felt something happening in his jaw, query tight.			Symptoms resolved without pharmocological treatment.

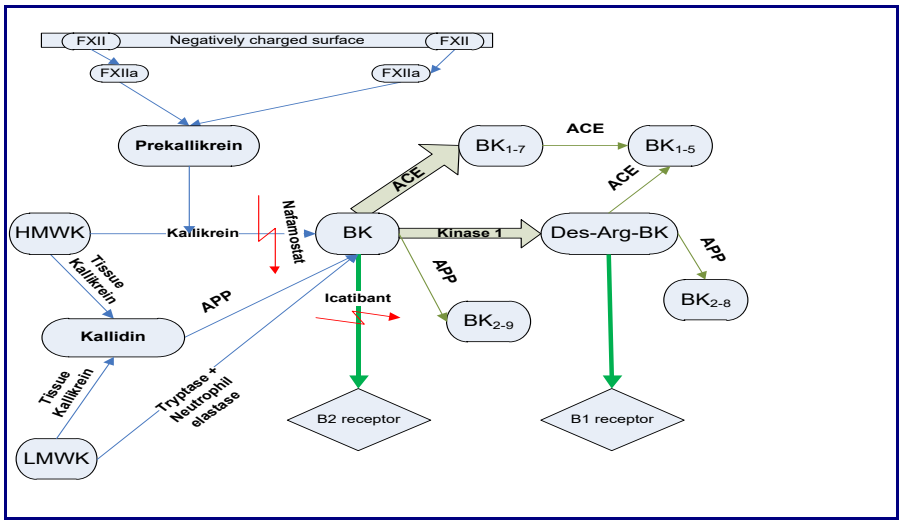

Figure 3. *Generation and metabolism of bradykinin. (Adapted from Hume HA et al 2012).*

sample and component labelling. Pre and post transfusion samples should have a group, antibody screen +/- antibody identification and direct antiglobulin test (DAT) +/- elution performed.

Our patient did not have fever, chills, shortness of breath, or show signs of bleeding. Visually the patient's urine showed no sign of haemolysis and the FBC, film and biochemistry profile (LDH, bilirubin, electrolytes and creatinine) did not indicate an increase in haemolysis. An AHTR appeared unlikely. It is noted that our patient has previously been transfused successfully with group O RhD Positive (OPOS) low titre apheresis platelets and the units implicated in these hypotensive reactions were both ABO compatible with our BPOS patient.

Transfusion related acute lung injury

TRALI is the occurrence of acute lung injury (ALI) within 6 h after the end of blood component transfusion, which is not related to other possible causes and risk factors of ALI (Goldman *et al* 2005). In 1994 the American-European Consensus Conference defined ALI as acute hypoxemia with a partial pressure oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) ratio of ≤300mm Hg, oxygen saturation (SpO_2) <90% on room air, bilateral pulmonary oedema on frontal chest radiograph and no evidence of left atrial hypertension. The mechanism of TRALI is complex and remains unclear, however it is believed to be immune mediated and comprise of a two-hit model where two separate events are needed for the clinical presentation. The first event involves specific patient clinical factors (e.g. infection, cytokine administration, recent surgery) which cause the activation of the pulmonary vascular endothelium and priming of neutrophils. This is thought to then lead to sequestration of neutrophils into the pulmonary microvasculature (Silliman *et al* 1997; Silliman *et al* 2005). The second event occurs via two different immunological triggers. The first is human neutrophil antigen (HNA) or HLA antibody in the donor plasma reacting with recipient neutrophils (HNA,HLA Class 1) or monocytes (HLA Class II) localised in the pulmonary microvasculature (Popovsky 1985; Sachs *et al* 2011). The HNA or HLA antibodies can also directly interact with HLA antigens of pulmonary endothelial cells (Dykes *et al* 2000). The second immunological trigger involves the accumulation of biologic response modifiers in the donor plasma which can prime polymorphonuclear cells leading to oxidative damage of the endothelium (Silliman 2003).

During the first severe hypotensive reaction our patient showed a decrease of SpO_2 from 96% to 89% 1 min after the transfusion started however did not report or display symptoms of respiratory distress such as dyspnoea or cyanosis. SpO_2 was immediately corrected with 2L nasal prong oxygen. Hypotension is a symptom of TRALI it is not normally as severe or as abrupt as noted with our patient. The underlying inflammatory pathogenesis of TRALI would also normally lead to a febrile reaction which was also not present in the first and second severe reactions. To our knowledge the patient did not have further radiological investigation for TRALI and we did not proceed with testing the patient and donor unit for HLA or HNA antibodies as part of the initial investigations. Subsequent testing performed independently of the reactions detected multispecific HLA Class I antibodies on the sample from 2/11/19 at 11:25 (pre reaction); however retesting on 3/11/19 post reactions (2/11, 3/11) did not detect these again. These antibodies can cause FNHTR and may be the cause of the temperature elevation during the third reaction 2/11/19 at 1820. Temperature increase was not noted in any of

the other reactions. The transient pattern of the antibody detection although potentially similar to a TRALI second event of inter-donor incompatibility described by Eastlund (1988), were not thought to be related to the patient's initial serve hypotensive reactions due to the speed of the reaction. The other clinical symptoms also did not include a febrile response or pulmonary oedema.

Transfusion-transmitted bacterial infections

In TTBI, the abruptness of symptoms following a bacterially contaminated unit is dependent on the bacterial species, the presence of endotoxins, the level of contamination and the recipient's immune status (Savage and Hod 2017). A study by Chui *et al* in 1994 presented data that concluded the onset of fever had a medium time of 1 h after transfusion for the septic group compared to 3 h for the febrile non-septic group. In a French study (Perez and Salmi 2001) reactions began within 15 min after starting transfusion of a unit containing Gram-negative bacteria, and 68 min after transfusion of a Gram-positive bacterially contaminated unit. A case study of septic shock during platelet transfusion in a patient with AML (Haesebaert *et al* 2013) also noted symptoms initiated within 15 min of beginning the transfusion. The Chui *et al* (1994) study however highlighted that the temperature increase is the key diagnostic feature in this type of reaction, with the mean temperature increase in bacteraemia patients of 2.6°C (SD±0.8) versus 1.8°C (SD±0.6) in the febrile non-septic group.

It is noted that our patient did not have an increase in temperature and therefore bacterial contamination was considered very unlikely. Blood cultures were not considered to be required by the treating clinician, laboratory haematologist or medical officer at the blood service. Blood cultures were however later requested by the treating clinician and returned a sterile result after 5 days.

Allergic, anaphylactoid or anaphylactic reactions

Mild allergic reactions occur in approximately 1%-3% of plasma transfusions (Stephen *et al* 1955; Kevy 1962) and severe anaphylactic reactions related to IgA deficient patients with anti-IgA range in occurrence from 1 in 20000 to 1 in 50000 (Pineda 1975). This makes these reactions the most common cause of severe hypotension as TRALI more often causes mild to moderate hypotension. Other studies report significant variation in incidence, reflecting different practices of pre-medication use, patient characteristics, product manufacture, storage time, reporting rates, reaction definitions and monitoring standards (Geiger and Howard 2007). Platelet transfusions are associated with a higher risk of allergic reactions compared to other components (Hirayama 2013). Mild to moderate allergic reactions as well as potentially life-threatening anaphylactic reactions are mediated by the production of immunoglobulin E (IgE) antibody in response to an antigen or allergen. Transfusion allergic reactions may be triggered by substances, usually proteins within the donor plasma, anticoagulants, preservatives or chemicals used in blood component manufacture, bag or infusion lines, or concomitant fluids and medications (Vamvakas 2012). Anaphylactoid reactions have the same symptoms as anaphylactic reactions however are not mediated by IgE antibodies. Both lead to the release of mast cell and basophil mediators (Tang 2003) and can be triggered by similar allergens.

Mast cells play a key role in allergic inflammation, as well as innate and adaptive immune responses.

The high-affinity IgE receptors, also known as FcεRI, or Rc epsilon R1 are implicated in allergic reactions. IgE antibodies are predominantly located in tissue bound to the FcεRI receptor on mast cells in their resting state. When antigen binds IgE, creating a crosslink between receptors, the mast cell becomes activated and degranulates, releasing both preformed mediators (including histamine, heparin, chondroitin sulphate, tryptase and chymase), and newly-synthesised mediators (including arachidonic acid metabolites and a number of chemokines and cytokines) (Janeway *et al* 1999; Migalovich-Sheikhet *et al* 2012).

Release of histamine, prostaglandin D2, leukotrienes C4 and D4 and tryptase, produces an immediate increase in local blood flow and increased vascular permeability, causing fluid to escape from capillaries into tissues, as well as smooth muscle contraction. This leads to the classic symptoms of allergy or type 1 hypersensitivity (watering eyes and nose, angioedema and hypotension). If the allergen is given intravenously, as is the case in blood transfusion, these reactions occur systemically leading to a catastrophic loss of blood pressure, constriction of the airways, and epiglottal swelling that can cause cardiovascular collapse and respiratory failure. Clinical severity is not only related to the route the allergen is introduced, but the amount of preformed allergen specific IgE antibodies present and the dose of the allergen (Janeway *et al* 1999). Symptoms of allergic, anaphylactoid or anaphylactic reactions that present following transfusion may be the most challenging to differentiate from an isolated hypotensive reaction. Understanding the pathophysiology behind these reactions may help identify variation in clinical presentations and target the appropriate management of the symptoms.

Initially allergic, anaphylactoid or anaphylactic reactions were considered as the most likely cause of the symptoms in our patient, with mild tightening of throat (potentially airway constriction) and flushing of face (potentially angioedema) in conjunction with the severe hypotension described. Elevated tryptase and reduced IgA levels were

also demonstrated in our patient. The tryptase results could not be clearly explained however could relate to his diagnosis of t-AML (Schwartz 2001; Schwartz 1987; Valent *et al* 2014). Other negative findings included prompt resolution of symptoms post-cessation of transfusion without other intervention, lack of other common allergy or anaphylaxis symptoms (listed in Table 4), negative testing for anti-IgA antibodies and multiple transfusions prior and afterwards with no reaction. Transfusion-related mechanisms responsible for inducing an allergic, anaphylactoid or anaphylactic response including both the introduction of the allergen or antibody into the recipient are outlined below.

Introduced allergens

Cases of IgA-deficient patients with pre-existing antibodies to serum immunoglobulin IgA are well documented, and commonly included in transfusion reaction investigation protocols. Antibodies can be directed to the IgA class or subclasses; IgA1, IgA2 or allotypes IgA2m(1), IgA2m(2). Antibodies directed to subclass specific determinants may explain why some patients with normal IgA levels may still develop allo-antibodies and subsequently suffer reactions post transfusion (Vyas *et al* 1969; Sandler *et al* 1995). Severe selective IgA deficiency may be diagnosed at levels of < 7mg/dL with normal immunoglobulin G (IgG) and immunoglobulin M (IgM) levels (Bonilla *et al* 2015; Conley *et al* 1993), however risk of formation of class specific anti-IgA is associated with absolute IgA deficiency (<0.05 mg/dL) (Vamvakas 2012; Sandler 1995; Eckrich 1993). Vyas *et al* (1969) showed 86% of patients with anaphylactoid reactions had anti-IgA antibodies of IgG class, with 75% relating to limited specificity or subclass antibodies. Forty four percent of patients with IgA deficiency had detectable anti-IgA antibodies, therefore although caution should be taken when transfusing an IgA deficient patient, it is not an absolute that they will make anti-IgA when exposed to plasma IgA. Schmidt *et al* (1969) highlighted that anti IgA antibodies can be naturally occurring therefore assumptions cannot also be made that the first transfusion will be tolerated.

Our patient had mildly decreased IgA levels, however not < 7mg/dL. Retrospective testing for anti-IgA by the blood service reference laboratory was negative for both anti-IgA1 and anti-IgA2.

Testing for haptoglobin levels and antibodies to this serum protein are also included in investigation protocols, however only a small number of cases have been reported. Koda *et al* (2000) determined the gene frequency for the homozygous deletion of the haptoglobin gene to be 1:1000 in Chinese, 1:1500 in Koreans, 1:4000 in Japanese, and not detected in African or European descent. Shimada *et al* (2003) also established that anaphylactic reactions to haptoglobin are prevalent in Japan with 6 out of 4138 non-haemolytic transfusion reactions related to anti-haptoglobin antibodies. Our patient's haptoglobin level was within normal limits at 0.45g/L.

The complement protein C4 is another allergen documented as a very rare cause of anaphylactic reactions (Weshoff *et al* 1992; Lambin *et al* 1984). Rodgers (Rg) and Chido (Ch) antigens are located on complement components C4A and C4B respectively which are then adsorbed onto red cells from plasma, making them the 17th blood group in the ISBT blood group systems list. If a recipient is either deficient in C4A (Rg-), C4B (Ch-) or both (null type) they have potential to make the red cell antibodies anti-Rogers and/or anti-Chido as well as IgG antibodies to C4 components (Reid *et al* 2012). Our patient had negative antibody screens throughout. Other potentially antigenic proteins include albumin, Von Willebrand factor (VWF) and other coagulation factors.

Transfusion of non-serum protein related allergens that the patients are pre-sensitized have been reported, although rarely. These include drugs e.g. penicillin (Michel 1980), chemicals e.g. methyleneblue (Dwachter 2011; Nubret *et al* 2011) food allergens e.g. seafood (Gao et el 2014) or peanuts (Jacobs 2011). We feel this mechanism is unlikely in our patient, given the patient has no known allergies and two different units caused reactions. It is not impossible, but unlikely that both the platelet units that the patient reacted to had donors with the same offending allergen, or sufficient allergen levels to trigger a reaction. The implicated donor units were also pooled units which have 70% plasma removed.

Introduced antibodies

The passive transfer of specific IgE antibodies has been described (Johnasson *et al* 2005), however the incidence of a large enough quantity to mount a significant allergic reaction coupled with the probability that the recipient is exposed to the particular allergen during or immediately post transfusion is a rare event (Cheung Yee Ching 2015). We believe this would not be the cause of our patient's reactions as identical hypotensive reactions occurred in two platelet units from two different donors, a day apart, and the symptoms occurred virtually immediately.

Acute isolated hypotensive reactions

The definition for AHyTR as per the International Society of Blood Transfusion (ISBT) working party on haemovigilance June 2013 is as follows:

A drop in systolic blood pressure of ≥30 mm Hg occurring during or within one hour of completing transfusion and a systolic blood pressure ≤ 80 mm Hg. Most reactions do occur very rapidly after the start of the transfusion (within minutes). This reaction responds rapidly to cessation of transfusion

and supportive treatment. This type of reaction appears to occur more frequently in patients on ACEi. Hypotension is usually the sole manifestation but facial flushing and gastrointestinal symptoms may occur. All other categories of adverse reactions presenting with hypotension, especially allergic reactions, must have been excluded. The underlying condition of the patient must also have been excluded as a possible explanation for the hypotension.

Reports of AHyTRs date back to the late 1970s (Alving *et al* 1978), where fractionated plasma products noted to contain high levels of prekallikrein activator (Hageman-factor fragments) triggered at least 23 patients in surgery to have reactions. Many of the early literature reports describe reactions occurring with the use of a negatively charged bedside leucocyte filter and it was later recognised to also correlate with the use of ACEi. The American Association of Blood Banks (AABB) Transfusion Practice Committee established it as a separate clinical entity in 1996 after a spike in acute hypotensive reactions in patients taking ACEi. In 2006, the ISBT working group and the European Haemovigilance Network (now the International Haemovigilance Network) introduced hypotensive transfusion reactions as a new category for haemovigilance systems.

Incidence rates for AHyTRs vary greatly across different international haemovigilance systems. This may be partly due to the different definitions in use or categorisation of hypotension symptoms relating to other reactions being included in the data for hypotensive reactions. Under-reporting due to clinical and laboratory staff not recognising the reaction is also highly likely.

The 2018 Serious Hazards of Transfusion (SHOT) report includes a single case of an isolated hypotensive reaction with a red cell unit. The 2018 report improves separation of the isolated hypotensive reaction, compared to previous reports that reported cases where hypotension was the predominant, not isolated, feature. In the 2010 SHOT report, which reported 19 cases of hypotension, the commentary included acknowledgement that the spike in hypotensive reactions reported included many patients undergoing major surgery or bleeding, and that it was unclear if haemorrhage was the true cause of hypotension.

To achieve a more accurate incidence rate, emphasis needs to be placed on the ISBT definition, with particular reference to eliminating other causes for the hypotension.

Central to the pathophysiology of the AHyTR is the low molecular weight bioactive nonapeptide BK and its active metabolite des-Arg9-Bradykinin (des-Arg9-BK). BK is cleaved from high molecular weight kininogens (HMWK) by plasma kallikrein and from low molecular weight kininogens (LMWK) by tissue kallikrein. Kallikrein (KK) is a kininogenase, which is a protease that specifically liberates kinins from kininogens (Hume and Robillard 2012; Bhoola *et al* 1992; Campbell 2013).

Circulating plasma HMWKs and prekallikrein (pKK) are synthesized in the liver and secreted in plasma. Physiologically, this system is involved in inflammation and blood coagulation with KK circulating in its somewhat inactive form pKK until required (Maurer *et al* 2011). Tissue-specific systems consist of locally synthesized or liver derived LMWK and tissue KK. Unlike plasma KKs, a continuous secretion of tissue KK can occur in organ-specific tissues, suggesting that tissue KKs contributes to physiological regulatory processes (Dendorfer *et al* 1998). BK is produced after the activation of the contact pathway of haemostasis. *In vitro* plasma contacting a negativelycharged surface (such as a leucocyte filter) initiates the contact system, which involves FXII (Hageman factor) and HMWK-Prekallirein complex binding to the negatively charged surface. Once bound FXII becomes activated (FXIIa) causing the generation of KK by cleaving pKK, which in turn cleaves BK from HMWK (Bhoola *et al* 1992; Campbell 2013).

BK has a short plasma half-life of only 15-53 s and circulating levels are usually relatively low (0.2–7.1pM) (Moreau *et al* 2007; Maurer *et al* 2011). BK is rapidly metabolized by endogenous metalloproteases including kininase II enzymes including angiotensin-converting enzyme (ACE), kininase I enzymes and aminopeptidase P (APP). ACE constitutes the main degradation pathway converting BK into inactive metabolites, however aminopeptidase P becomes the major BK degradation enzyme when an ACEi is present. The relevant metabolic pathways are shown in Figure 3.

BK exerts its potent vasodilatory effects on B2 receptors, whereas des-Arg9-BK exerts its effects on B1 receptors (Bhoola *et al* 1992; Holdstock 1957; Campbell 2013). B2 receptors are constitutively expressed in vascular endothelium, and are responsible for local and systemic haemodynamics e.g. decreased blood pressure, due to vasodilation and vascular permeability, smooth muscle contraction e.g. bronchospasm, bronchoconstriction, cell proliferation and migration, pain via stimulation of sensory nerves, and possibly angiogenesis (Vianna *et al* 1998; Dlamini *et al* 2005; Bandeira-Melo *et al* 1999; Giusti *et al* 2005; Maurer *et al* 2011). BK exerts it vasodilatory effects by binding to the B2 receptors on endothelial cells leading to the release of nitric oxide, prostacyclin, elevated intracellular Ca2+ and endothelium-derived hyperpolarising factor (Maurer *et al* 2011). B1 receptors are inducible with expression up-regulated by tissue injury and inflammation, by cytokines, endotoxins and growth factors, and have been shown to be involved in BP homoeostasis, promoting neovascularization and angiogenesis (Hume and Robillard 2012; Giusti *et al* 2005; Maurer *et al* 2011).

Once BK binds to the B2 receptor it mediates a rapid desensitizing response where the receptor undergoes

endocytosis and recycling to the cell surface. In contrast des-Arg9-BK binding B1 interrupts receptor recycling and stabilises the receptor leading to longer lasting receptor signalling (Maurer *et al* 2011). Bandeira-Melo *et al* (1999) studied the role BK has in mediating allergic inflammation via the suppressive effects of distinct BK B2 receptor antagonists. They established that BK acting on its B2 receptors induce mast cell degranulation in both sensitised and non-sensitised rats, proving BK in the absence of allergy can activate mast cells. This supports our theory that an allergic reaction was not necessary to cause an elevated tryptase in our patient.

Tryptase acts as a kininogenase and increases release of BK from HMWK and LMWK. It was initially questioned whether the high tryptase level detected acted independently of BK to produce the post transfusion symptoms, or whether the assumed increased BK levels (reported to cause acute hypotensive reactions) caused the tryptase elevation demonstrated. If direct BK stimulation in the absence of antibody mediated allergy can lead to significant mast cell degranulation and therefore tryptase and histamine release, it can be suggested that the tryptase assay is not adequate to differentiate between a true anaphylactic reaction and an AHyTR. The results from the Bandeira-Melo *et al* (1999) study and the knowledge that tryptase increases BK levels may highlight that BK has an important feedback role to amplify the reaction in allergic processes.

BK degrades quickly and has a limited dose provided to the patient at the time of transfusion via the stored plasma or a spike produced by a bedside leucocyte filter. This is possibly why the transfusion reaction in some people is limited to hypotension. Larger doses or continued stimulus from BK may potentially expand the symptoms detected to replicate a standard anaphylactic reaction, taking longer to resolve.

Our patient presented with a severe and instant drop in blood pressure within minutes of the transfusion starting without clear signs of significant respiratory distress or fever followed by a rapid resolution, which would be consistent with a reaction mediated by a short-lived vasoactive substance.

Compounding effects

The rarity of AHyTR is likely due to the need for multiple compounding effects to reach a particular threshold. These may include transfusion generation of BK, activation of FXII and generation of KK including the influence of negatively charged leucocyte filters (Takahashi *et al* 1995; Hild *et al* 1998; Abe *et al* 1998), injury or surgical tissue damage causing increased B1 receptor expression (Hume and Robillard 2012; Giusti *et al* 2005; Maurer *et al* 2011), release of tissue KK as described in the setting of prostatectomy (Arnold *et al* 2004; Lilja *et al* 1999) and cardiopulomary bypass (CPB) (Cugno *et al* 2001), and impaired BK metabolism due to CPB, ACEi or genetic abnormalities in ACE or APP levels (Shiba 2003). We confirmed none of our patient's transfusions used a negatively charged filter.

Patients taking ACEi (Table 5) can suffer hypotension and angioedema. These are more commonly reported on commencement of the drug but can also occur when increasing the dose, during surgery, during apheresis procedures and rarely during non-surgical transfusion. Shiba and colleagues (2003) studied the effects of BK metabolism in the transfusion recipient and reported that only recipients with intrinsically low levels of ACE activity demonstrated significant increases of plasma BK levels post transfusion. The individual's ability to metabolise a transfusion-associated increase in BK is potentially as important as the quantity of BK infused in the donor plasma. ACEi inhibits ACE the main degradation pathway for BK. A recent discovery indicates ACEi directly binds to B1 receptors leading to nitric oxide release and vasodilation (Ignjatovic *et al* 2002; Kugaevskaia and Eliseeva 2011). ACEi use in the absence of transfusion is noted to have many side effects (Table 6) and are similar to many of the symptoms our patient suffered (Morimoto *et al* 2004; Dicpinigaitis 2006; Beltrami *et al* 2011). Donor BK kinetic variation has also been described (Hild *et al* 1998; Moreau *et al* 2007). Moreau and colleagues (2007) showed that during storage, after an initial rapid (<1hr) degradation, levels of BK again began to rise and also peaked (> 1ng/ml in 22% of units) at 5 days post filtering. As there are no practical ways to measure BK or des-Arg9-BK in donor plasma there is potential for some donor units to trigger a reaction in susceptible patients. Freshly prepared platelet units may limit the occurrence of hypotensive reactions.

The exact level of kinins needed to cause a hypotensive reaction is not confidently known. In a study by Bonner *et al* (1990) a dose of 0.8-1.2 µg/kg body weight was needed to achieve a hypotensive effect in healthy humans however ACEi potentiate the blood pressure-lowering effects of BK dose approximately 20-50 fold. This dose limit does not fit with the concept that the donor unit is the main source of BK, as in our experience our patient only received a very small volume before triggering a reaction. Therefore we propose that our patient's own plasma level of kinins was elevated significantly before transfusion and the unit may have been the tipping point.

Testing available for markers of reaction

Differentiating an allergic reaction from an AHyTR may be difficult as the initial manifestations may be the same. The potentiator of the reaction in AHyTR is however a non-immunological source and finite in ability to stimulate ongoing reaction. Markers that may be increased in both include histamine, tryptase or BK levels.

Table 2. *Products provided prior, during and post transfusion investigation period*

Date of transfusion	Donor unit blood group	Product type	Quantity
15/09/2019	OPOS	Platelet apheresis	1
15/09/2019	BPOS	Red cells	1
16/09/2019	OPOS	Platelet- apheresis	1
16/09/2019	OPOS	Red cells	1
18/09/2019	BNEG	Red cells	2
19/09/2019	OPOS	Platelet-pooled	1
20/09/2019	ONEG	Red Cells	1
22/09/2019	BPOS	Platelet-pooled	1
23/09/2019	BPOS	Platelet-pooled	1
23/09/2019	BPOS	Red cells	2
25/09/2019	BPOS	Platelet- pooled	1
27/09/2019	OPOS	Platelet- apheresis	1
***29/09/2019**	BPOS	Platelet-pooled	1
***30/09/2019**	BNEG	Platelet- pooled	1
2/10/2019	BPOS	Platelet- pooled	1
3/10/2019	BNEG	Platelet- pooled	1
4/10/2019	OPOS	Red cells	1
26/10/2019	ONEG	Red cells	1
28/10/2019	BPOS	Red cells	1
30/10/2019	OPOS	Red cells	2
1/11/2019	OPOS	Platelet- apheresis	1
***2/11/2019**	BPOS	Platelet-pooled	1
***2/11/2019**	BNEG	Red cells	1
***3/11/2019**	BPOS	Platelet- pooled	1
5/11/2019	BPOS	Red cells	1
*** unit implicated in suspected transfusion reactions.**			

Table 3. *Laboratory results over the investigation timeframe*

Laboratory results	Reference Range	Date/ time of collection													
		23/09/19 05:30	28/09/19 07:00	29/09/19 16:35	30/09/19 05:15	30/09/19 17:30	01/10/19 15:50	02/10/19 0500	03/10/19 01:00	03/10/19 09:05	01/11/19 1436	02/11/19 11:25	02/11/19 19:15	03/11/19 16:15	05/11/19 0655
Blood group		BPOS	–	–	–	–	–	–	–	–	–	*BPOS	BPOS	–	–
Antibody screen		Negative	–	–	–	–	–	–	–	–	–	*Negative	Negative	–	–
DAT		–	–	–	–	–	–	–	–	–	–	Negative	Negative	–	–
Visual inspection for haemolysis-plasma		–	–	–	–	Nil	Nil	Nil	–	–	–	Nil	Nil	Nil	Nil
Visual haemolysis or turbidity-unit		–	–	Nil	–	Nil	–	–	–	–	–	–	Nil	Nil	–
Visual haemolysis – urine		–	–	Nil	–	Nil	–	–	–	–	–	–	Nil	Nil	–
Haemoglobin	125-175 g/L	77	86	99	82	86	81	80	–	81	86	81	113	–	74
WCC	3.5-10.0 x10^9/L	0.2	0.2	0.2	0.2	0.2	0.2	0.3	–	0.3	1.4	0.4	0.4	–	<0.1
Platelet count	150-400 x10^9/L	18	30	13	10	11	8	<5	–	9	11	15	18	–	5
Film review: polychromatic cells agglutination or spherocytes (Y/N)		N	N	N	N	N	N	N	–	N	N	N	N	–	N
Sodium	135-145 mmol/L	140	139	–	141	142	142	141	–	138	142	–	137	–	138
Potassium	3.5-5.5 mmol/L	3.7	3.6	–	3.4	3.1	3.0	3.7	–	3.0	3.5	–	3.2	–	3.6
Chloride	95-110 mmol/L	104	107	–	107	106	104	103	–	101	107	–	103	–	102
Urea	3.5-9.0 mmol/L	5.5	4.5	–	5.9	4.6	3.5	4.2	–	4.1	6.9	–	6.0	–	5.1
Creatinine	60-110 µmol/L	72	68	–	70	68	62	69	–	61	81	–	70	–	70
eGFR	>59	>90	>90	–	>90	>90	>90	>90	–	>90	86	–	>90	–	>90
Total bilirubin	<21 µmol/L	18	16	–	14	17	14	16	–	15	28	–	37	–	40
LDH	120-250 U/L	220	158	–	133	145	133	130	–	143	177	–	153	–	103
Troponin	<26 ng/L	–	–	–	–	–	–	–	–	–	–	–	–	<2	–
Blood cultures		–	–	–	–	–	–	–	Sterile	–	–	–	4 x BCs sterile	–	Sterile
IgA	1.24-4.16 g/L	–	–	–	–	–	–	–	–	–	–	–	–	0.71	–
anti-IgA		–	–	–	–	–	–	–	–	–	–	Negative	–	Negative	–
Tryptase	<13.5 µg/L	–	–	20.6	–	19.2	–	–	–	12.9	8.6	–	–	8.0	–
HPA genotyping		–	–	–	–	–	–	–	–	–	–	1aa 2aa 3ab 5aa 15aa	–	–	–
HLAgenotyping		–	–	–	–	–	–	–	–	–	–	A*03; B*07; C*07	–	–	–
Platelet antibody (PAK-Lx and MAIPA)		–	–	–	–	–	–	–	–	–	–	Negative	–	Negative	–
HLA antibody (LabScreen Class 1 single antigen)		–	–	–	–	–	–	–	–	–	–	‡ Positive	–	Negative	–

Table 4. *Classification of hypotensive transfusion reactions*

Reaction classification	Incidence	Most common product implicated	Signs and symptoms
Immune mediated			
Anaphylaxis	1:20,000-1:50,000	Platelets and plasma	*Symptoms can depend on route of allergen introduction; however transfusion reaction usually produces a systemic response.* **Cardiovascular:** Hypotension (severe), loss of consciousness and shock,tachycardia, cardiac arrest. **Cutaneous**: Pruritis, urticaria, erythema, flushing, angioedema. **Respiratory:** Upper airway obstruction (hoarseness, stridor, "lump" in throat), lower airway obstruction /bronchospasm (wheezing, chest tightness, substernal pain), dyspnoea, cyanosis, anxiety, feeling of impending doom. **Gastrointestinal:** Nausea, vomiting, abdominal cramps, diarrhoea.
TRALI*	1:1200 -1:190,000	Plasma	Hypotension (mild to moderate), respiratory failure, hypoxia, cyanosis, fever, bilateral pulmonary oedema
Acute Haemolytic Transfusion Reaction (AHTR)	1:76,000, fatal in 1:1.8 million	Red Cells	Hypotension (none to severe), tachycardia, dyspnoea, anxiety, fever, chills or rigors, pain (back, chest and infusion site), haemoglobinuria, renal failure, oligouria, haemorrhage (DIC), nausea and vomiting.
Non immune mediated			
Transfusion Related Sepsis	Platelets: 1:100,000, fatal in 1:1,000,000 Red cells: 1:500,000 fatal in 1:10,000,000	Platelets	Hypotension (mild to severe) +/- tachycardia, fevers, chills or rigors, nausea and vomiting, dyspnoea or in severe cases circulatory collapse, renal failure or DIC.
Acute Hypotension associated with ACE Inhibitors	Limited data, not well established. 0.66:100,000 – 6.8:100,000	Platelets	Hypotension (severe) often isolated, occasionally flushing, pain, mild respiratory symptoms (dyspnoea, ↓SpO2, cough/throat irritation),+/- gastrointestinal symptoms.
Air embolism	Rare	Rapid infusion and blood	Hypotension, sudden shortness of breath, acute cyanosis,shoulder and back pain, cough, cardiac arrhythmia.

Histamine has a significant role in inflammation during the late phase allergic response, however in relation to transfusion reactions it is the immediate response after IgE or BK mediated mast cell activation that is of most importance. Early diagnosis can be achieved by plasma histamine measurements. Unfortunately, the short plasma half-life of histamine and the difficulties in handling the sample usually preclude this measurement, although a sensitive radioimmunologic kit is routinely available (Laroche *et al* 1991).

Laroche *et al* (1991) investigated feasibility of measuring tryptase instead of histamine as a biochemical marker of anaphylaxis. They determined that tryptase half-life was equal to 90 mins and at least 15 mins was necessary to reach the peak level after a reaction. The best time for measuring tryptase was 1-2 h after the reaction (not greater than 6 h). They concluded that measurement of plasma tryptase along with measurement of plasma histamine may aid in diagnosis of anaphylaxis.

Tryptase is commonly requested in the investigation of possible allergic or anaphylactic reactions. Limitations include long turnaround time as it is a specialised test sent to larger laboratories. This test is therefore only useful for retrospective confirmation of the cause. Tryptase peaks at around 1-2 h following the reaction and returns to baseline within 24-48 h except in the case of renal insufficiency delaying clearance (Gotlib *et al* 2018). Ideally a baseline pretransfusion tryptase should be tested and compared with the sample immediate post transfusion as underlying reasons for elevated tryptase may complicate the diagnosis. Other conditions that may increase tryptase include systemic mastocytosis, chronic helminth infections, mast cell hyperplasia, stem cell factor treatment, and myeloid neoplasms (MDS, MPN, AML, CEL with FIP1L1-PDBFRA fusion gene) (Schwartz 2001; Schwartz 1987; Valent *et al* 2014). A significant increase in tryptase is expected in anaphylactic reactions.

There seems to be a poorly defined association between elevated tryptase and patients suffering acute reactions (angioedema and hypotension) when taking ACEi. Rasmussen *et al* (2017) reported 105 out of 734 patients assessed due to angioedema were found to have ACEi-induced angioedema (14%), and of these, 5.7% had an elevated tryptase and 2.9% had a positive histamine release test at time angioedema was present, without signs of allergic rhinitis, asthma or atopic dermatitis. None of the patients with recurrent angioedema after ACEi withdrawal had a positive histamine-release test or elevated tryptase.

Sprung *et al* (2015) reported on a case of refractory intraoperative hypotension with elevated serum tryptase likely associated with lisinopril therapy. The authors excluded anaphylaxis or mastocytosis as the source of elevated tryptase with a negative urine N-methylhistamine and 11-β prostaglandin F2α, negative D816V c-kit mutation and absence of allergic clinical features (urticaria, bronchospasm etc.). The proposed reason for elevated tryptase in this case was renal insufficiency. It is noted that our patient did not show signs of renal insufficiency; however was undergoing induction chemotherapy for t-AML at the time of the first two reactions (likely the cause of elevated tryptase level prior to any reactions).

Measurement of BK or its metabolite des-Arg9-BK is theoretically possible by mass-spectrometry, however it is currently restricted to research laboratories and limited by the very short half-life of 15 s. It is not well described what the exact threshold for BK or des-Arg9-BK levels are to trigger hypotensive symptoms in a patient, and there are no published definitive BK or des-Arg9-BK reference ranges for patients on ACEi available.

Management and prevention

1. Stop the transfusion

As with all transfusion reactions the first action should be to immediately stop the transfusion. In the case of AHyTR this is often all that is required and BP will spontaneously increase within minutes after ceasing the transfusion as the BK B2 receptors desensitise and BK continue to be metabolised even in the presence of ACEi. If hypotension is severe or unresponsive to fluids then vasopressor therapy may be necessary (Sahu *et al* 2014; Savage *et al* 2017; Tang 2003). The possibility of acute haemolytic or septic reactions must be considered in all cases and TRALI or allergic reactions should be excluded if other symptoms of these reactions appear.

2. Slow the infusion rate of blood products

As BK has a very short half-life and the reaction is predicted to be caused by the sudden spike of BK delivered by the transfusion, reducing the infusion rate may assist.

3. Consider discontinuation of ACEi treatment

Many patients who receive transfusions are on ACEi and they do not experience adverse reactions, therefore routine modifications to dosing or ceasing the drug completely is not justified. In patients that have shown to repeatedly suffer severe hypotensive reactions whilst on ACEi, weighing up the risk of changing the anti-hypertensive medication, or interrupting the dosing at least 24 h to several days prior to the transfusion, depending on type of ACEi (Hume *et al* 2012), could be considered.

4. Avoid negatively charged bedside filters

Several studies have reviewed the increased generation of BK, activation of FXII and generation of KK when blood

products are passed through a negatively charged leucocyte filter (Takahashi *et al* 1995; Hild *et al* 1998; Abe *et al* 1998). Although many manufacturers now provide pre-storage leukocyte reduced units, clinical staff should be alert to the risk of using negatively charged leucocyte filters during transfusion, apheresis or cell salvage procedures and consideration should be given to either not using such filters, or using a different filter.

5. Consider washed cellular components

Washed cellular components have been shown to be effective for preventing allergic transfusion reactions by removing the offending allergen or biological response modifiers (BRMs) in the donor plasma (Buck *et al* 1987; Blumberg *et al* 2004). It should be noted that washing of platelet units may occasionally be ineffective as BRMs and IgA are internalised in platelets and can be released into the unit post washing, during storage or post transfusion (Hirayama 2012; Herter *et al* 2014; Sloand *et al* 1990; Griswold 2014). The washing process will reduce accumulated BK, histamine and neutrophil priming lipids which are all suspected of triggering hypotensive or allergic symptoms. Washing cellular components however has several limitations. It adds significant cost to unit production, is time consuming, delays transfusion, may present additional opportunity for bacterial contamination, causes loss of substantial numbers of platelets during the washing procedure and platelet activation and platelet recovery after transfusion may not be equivalent to that with unwashed platelets (Blumberg *et al* 2004; Hirayama 2012; Schoenfeld 2004). Another significant limitation to washed platelet units is the very short expiry post washing which limits supply to regional facilities.

6. Consider the use of premedications

General use of common transfusion premedications (anti-histamines, hydrocortisone or acetaminophen), that target allergic or inflammatory reactions is a controversial topic where some studies show they are not efficacious in preventing a reaction and could potentially cause harmful side effects (Ning *et al* 2019; Kennedy *et al* 2008; Brown 1998). Premedication was shown to be not effective for our patient.

7. Emerging novel therapies

Endothelial B1 and B2 receptors and elevated BK are implicated in many clinical situations including hypotension and angioedema. It appears feasible, therefore, that inhibitors of BK production (KK inhibitors) or receptor binding (B1 and B2 antagonists) might deliver clinical benefit for hypotensive patients. Nafamostat mesilate has KK inhibiting activity and has been successfully used to inhibit BK generation in low density lipoprotein (LDL) apheresis and CPB (Kojima *et al* 1991) and Icatibant, a B2 receptor inhibitor is approved for treatment of hereditary angioedema, a condition of increased production of BK with normal degradation pathways. However in a recent randomised trial Sinert *et al* (2017) found Icatibant provided no improvement to angioedema induced by ACEi.

Conclusion

AHyTR, although rare, may be under-reported. This is likely due to the isolated symptom either being considered non-significant to report due to quick resolution, or thought to not be directly related to the transfusion as the reaction workups are negative for serological or bacterial causes, which are the more common causes of transfusion related hypotension.

This review highlights the complexity and overlap of presenting symptoms in acute transfusion reactions and the benefits of considering the predominant signs or symptoms as a guide for further investigation and management. Confirmation of a diagnosis of AHyTR may involve exclusion of all other aetiologies however, without the clinical team recognising this type of reaction and the potential for a transfusion-related source, appropriate investigations to establish a link to the transfused product in conjunction with the patient's ACEi will not be instigated, perpetuating the cycle of under recognising this clinical entity. In addition it is important for laboratory scientists and pathologists to be aware of this as a potential transfusion reaction and correlate with the medication history when presented with a hypotensive reaction.

With the evidence suggesting AHyTR in patients on ACEi involve non-IgE-mediated mast cell activation it could be considered a subset or first stage of an anaphylactoid reaction. Our review suggests an elevated tryptase following an AHyTR cannot be used to definitively determine an allergic or anaphylactic cause in a patient on ACEi therapy, and that clinical history including medication history is of primary importance in the initial assessment of a possible AHyTR.

Table 5. *ACE inhibitor drug examples*

Drug proprietary name / Generic name
Epaned / Enalapril
Monopril / Fosinopril
Capoten / Captopril
Univasc / Moexipril
Altace / Ramipril
Accupril / Quinapril
Lotensin / Benazepril
Vasotec / Enalapril
Prinivil / Lisinopril
Zestril / Lisinopril
Qbrelis / Lisinopril
Mavik / Trandolapril
Aceon / Perindopril

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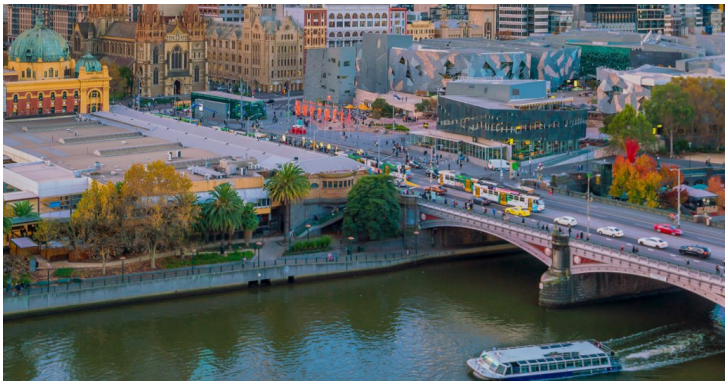

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Microplastics in pharmaceutical dosage forms and patient informed consent

Nial J. Wheate[1]

[1]*School of Pharmacy, Faculty of Medicine and Health, The University of Sydney, Sydney, NSW*

Abstract

Microplastics are approved ingredients for inclusion in specific pharmaceutical dosage forms in Australia. Polystyrene sulfonate is a synthetic polymer that is used either as the active pharmaceutical ingredient or as a sustained release excipient in pharmaceutical products. Current scientific evidence shows that microplastics, especially those that are styrene sulfonate-based, are a hazard to both the environment and human health. Microplastics can enter the environment through their inclusion in consumer products (e.g. body washes), pharmaceutical products, and through the degradation of plastic waste. In fresh- and salt-water systems, the microplastics affect the health of aquatic animals, and through bioaccumulation in the food chain results in their ingestion by humans from the consumption of seafoods such as molluscs and shellfish. There is considerable evidence that microplastics can be absorbed and persorbed from the gastrointestinal tract. This can cause life-threatening colonic necrosis and a range of other conditions such as bowel wall ulceration and perforation, abdominal pain, distension, nausea and vomiting, gastrointestinal bleeding, diarrhea, embolism of small vessels, genotoxicity, oxidative stress, and cellular apoptosis and necrosis. Microplastic can also be aspirated in the lungs causing bronchitis and pneumonitis and can accumulate in organs such as the liver. Currently the information provided to patients and health staff is need of revision so that patients can provide informed consent on their use and manufacturers need to be compelled to make clear statements on their packaging that their pharmaceutical product contains microplastic particles.

Keywords: Microplastic, styrene sulfonate, drug delivery, pharmaceutical formulation, safety

Introduction

Microplastics, in the form of microbeads, are permissible ingredients in selected pharmaceutical dosage forms. They can be used as the active pharmaceutical ingredient (API) or as pharmaceutical excipients for a range of applications that improve the delivery of API molecules. The plastics in pharmaceutical products are in the form of ion-exchange resins. These are high molecular mass polymers that can exchange ions of equal charge with gastrointestinal body fluids (Yoshida *et al* 2013). Some contain negatively charged sites on the surface and pores of the bead if needed to deliver cationic API molecules. Conversely, positively charged microplastics can be used to deliver anionic API molecules, but these are not common.

There are three primary applications for the inclusion of microplastics as potassium exchangers, for the sustained release of API molecules, and taste masking (Yoshida *et al* 2013). Examples of pharmaceutical products that contain microplastics are given in Table 1. Hyperkalaemia is defined as a patient having a high level of potassium in their blood serum that is well above normal levels (Palmer and Clegg 2017). Because of the ability of potassium to affect the heart, severe hyperkalaemia is considered a medical emergency in many situations (Kovesdy 2015). Many countries, including Australia, have approved specific microplastic-based medicines for the treatment of hyperkalaemia. These medicines contain anionic styrene sulfonate microplastics bound with either sodium or calcium cations. When swallowed by the patient, the sodium/calcium cations are exchanged over a period of days or weeks with potassium, thus removing the excess potassium (Chaitam *et al* 2016).

There are a number of alternative treatment options available which include stopping the use of medicines that may increase potassium levels, dietary changes, dialysis, and the use of potassium exchangers (Kovesdy 2015).

The most common application of microplastics is for the sustained release of API molecules (Anand *et al* 2001). The drugs are absorbed onto the surface of the microplastics where they are held in place through ionic bonds and

Address correspondence to:
Nial J. Wheate
School of Pharmacy, Faculty of Medicine and Health,
The University of Sydney,
NSW, 2006, Australia
E-mail: nial.wheate@sydney.edu.au

Table 1. *Example pharmaceutical dosage forms that contain microplastics as either the API or as an excipient for the improved delivery of the API molecule.*

Medicine	API	Plastic	Condition/Use
Dyanavel XR	Amphetamine	SPS*	ADHD
Penntuss	Phenylephrine/ Chlorpheniramine	Polistirex	Allergies
Delsym	Dextromethorphan	Polistirex	Cough
Betoptic® S	Betaxolol	HPS*	Glaucoma
Resonium A®	SPS	SPS	Hyperkalaemia
Duromine™	Phentermine	SPS	Weight loss

**SPS, sodium polystyrene sulfonate; *HPS, hydrogen polystyrene sulfonate.*

potentially through hydrophobic effects between the plastics' backbone and lipophilic regions of the APIs.

When swallowed by the patient, the low pH of stomach fluids (typically around 1.5)(Beasley *et al* 2015) means the high hydrogen ion (H^+) content results in the slow release of the API through an ion-exchange effect.

Many API molecules have a bitter taste which reduces acceptability to patients and can lead to non-compliance with medications (Mennella *et al* 2013). A third application of microplastics is therefore as a taste-masking excipient in pharmaceutical dosage forms (Dugad *et al* 2018). Similar to the formulation of the sustained-release products, bitter tasting API molecules are absorbed onto the surface of the microplastic particles. The relatively neutral pH of saliva (Baliga *et al* 2013) means the drug remains bound to the plastic in the mouth and is only released upon contact with stomach fluids.

Microplastics are also being investigated for additional applications in pharmaceutical dosage forms. There is interest in using microplastics to aid with the solubility of API molecules, as coatings on metallic nanoparticles for drug delivery (Li *et al* 2020), and to reduce hygroscopicity (Shang *et al* 2018). While no products are Australian Therapeutic Goods Agency (TGA) listed on the Australian Register of Therapeutic Goods (ARTG) for those specific applications, it is possible that new products may be developed with subsequent applications to the TGA for marketing approval. A range of different microplastics are available depending on the purpose of the pharmaceutical product and the desired physical characteristics. The type of acid/base functional group, size and size distribution of the particles, and the degree of cross-linking can all be varied to tune the microplastics for a specific API (Guo *et al* 2009). The most commonly used plastic for the delivery of cationic API molecules are those based on styrene sulfonate, with divinylbenzene (DVB) sometimes included for its cross-linking of the polymer chains.

Polystyrene sulfonate

A styrene sulfonate-based microplastics are the most used in pharmaceutical dosage forms. Monographs for sodium polystyrene sulfonate (SPS) are provided in both the United States Pharmacopeia (Inc 2017) and the British Pharmacopeia (Commission 2016).

Amberlite™ IRP69 is an example of a styrene sulfonate-based microplastic. This plastic is the sodium salt of polystyrene sulfonate and is sold as a resin for use in pharmaceutical applications. Specific applications listed in the specification include taste masking, drug stabilisation, sustained release, and as the active ingredient for potassium exchange. It contains both styrene sulfonate and DVB residues throughout the polymer chains.

The use of styrene sulfonate as an ingredient in pharmaceutical dosage forms was first approved by the US Food and Drug Administration (FDA) in 1958 (Chaitam *et al* 2016). As both an API and excipient, styrene sulfonate microplastics are included as an ingredient for the products Duromine™, Betoptic® S, and Resonium A® on the ARTG. The calcium form of the plastic is used in the product Resonium C® which is also listed on the ARTG, and the non-salt form of SPS, polystyrene sulfonate hydrogen (HPS) are used in Bitoptic® S products.

Important to the use of microplastics in pharmaceutical dosage forms are their size and shape. The size of SPS microplastics are typically within the range of 11 to 124 microns (Zann *et al* 2017), with 10 to 25% of SPS particles expected to have a size greater than 75 microns, and up to 1% of the particles may have a size greater than 150 microns. This means that the majority of SPS particles are

skewed to the smaller sizes, which unfortunately facilitates their uptake by the human body. Styrene sulfonate microplastics are also reported to be irregularly shaped (not round) which gives them a poorly rated mouth feel and texture (Zann *et al* 2017). An irregular shape is important as pointed edges can help the particles become trapped in the lining of the gastrointestinal tract, which could facilitate their unintended uptake by the body.

Of particular note for styrene sulfonate-based microplastics is their reported hazard rating (Lithner *et al* 2011). Using the UN Globally Harmonized System, styrene-based plastics have been assigned as a Class V hazard; the highest hazard rating on their five-point scale. In this rating systems, a class V hazard plastic is a class 1A/1B carcinogenic, a class 1A/1B mutagenic, and class 1A/1B for reproductive toxicity. The hazard rating for plastics is based on its chemical composition and the monomer from which it is manufactured (Lithner *et al* 2011). A class/category 1A chemical is one that is broadly known to have carcinogenic/mutagenic/reproductive toxicity potential for humans (the placing of a substance in category 1A is largely based on human evidence) while a category 1B chemical is presumed to have carcinogenic/mutagenic/reproductive potential for humans (the placing of a substance in category 1B is largely based on animal evidence).

In 2018, the FDA also acknowledged the carcinogenic potential of styrene, and as a result, no longer permits its use as a food additive (FDA).

Environmental hazards

Microplastics can turn up in the environment in one or two ways; from consumer goods and pharmaceutical products, and from the degradation of larger plastics (Bouwmeester *et al* 2015). They can contaminate both fresh and salt-water systems (Klein *et al* 2018), and in 2015 it was calculated that per day, eight trillion pieces of microplastic, in the form of microbeads, were emitted into the aquatic environment of the Unites States alone (Rochman *et al* 2015). This has been calculated in an Australian study to result in up to 14 million tonnes of microplastics on the ocean floor (Barrett *et al* 2020). There is now evidence that it is possible that microplastics can be degraded into nanoparticles by small crustaceans (Mateos-Cárdenas *et al* 2020).

The accumulation of plastics and microplastics in the environment is an acknowledged concern both in Australia and the rest of the world. There is clear guidance from the Australian Government regarding the impact of microplastics on the environment from their statement that “once in the water, microbeads can have a damaging effect on marine life, the environment and human health” (Australian Government 2020). The Australian Microplastic Assessment Project states that “of the numerous recognised impacts to wildlife from marine litter, over 70% can be attributed to microplastics, and with their ability to move through the food chain, effects are compounded” (Australian Microplastic Assessment Project 2020). It is known that microplastics can promote pathogen transmission between animals, that they can reduce the feeding capability of corals, that they are able to penetrate the cell walls of plankton and affect their chlorophyll (the chemical they use to convert carbon dioxide to oxygen), and that microplastics can affect the blood chemistry of fish (Chatterjee and Sharma 2019).

With regard to styrene-based microplastics, they have been shown to be able to affect the hatching of fish eggs (making them hatch later) with smaller and slower larvae (Chatterjee and Sharma 2019). Styrene-based microplastics have also been shown to have a toxic effect on the livers of fish (Lu *et al* 2016). Microplastics in the environment can accumulate heavy metals on their surfaces such as cadmium, zinc, nickel, and lead (Wright and Kelly 2017) which can be released at a later time. Finally, microplastics are also capable of leaching their constituent monomers into the environment (Galloway 2015).

These problems are important because microplastics can be accumulated in the food chain (Wagner *et al* 2014) specifically, microplastic bioaccumulation by marine animals that are consumed as food by humans. Release of heavy metals and leaching of plastics monomers can therefore put people at risk who have never previously used any product containing a microplastic.

In addition to pharmaceutical products there are a number of consumer products that contain microplastics. Examples includes body scrubs, deodorants, toothpastes, sunscreens and cosmetics. In such products, the plastics are in the form of microbeads that are small, solid manufactured plastic particles with an upper diameter limit of 5 mm, but with typical diameters of around 100-300 microns. This makes the microplastics used in consumer products very similar to the microplastics used in pharmaceutical products. The environmental impact of microplastics is acknowledged in Australia and in other countries. Due to this impact, many countries have acted to remove them from consumer products. In 2017, The Environmental Protection (Microbeads) (England) Regulations were enacted which makes it illegal to manufacture or sell rinse-off consumer products that contain plastics in microbead form.

A similar law was passed by the United States with the enactment of the Microbead-Free Waters Act of 2015. This legislation makes the manufacturing, packaging, and distribution of rinse-off cosmetics containing plastic microbeads illegal. Importantly, the law also applies to products that are both cosmetics and non-prescription medicines, such as toothpastes.

In Australia, there is no legislation banning the use of plastics in microbead form, but the federal Department of Agriculture, Water and the Environment has instead introduced a voluntary industry phase-out of solid plastic microbeads from rinse-off personal care, cosmetic and cleaning products. This initiative was announced based on the recommendations of the NSW Environment Protection Authority's NSW Microplastics Working Group.

In 2018, the Department of the Environment and Energy conducted an assessment of the phase-out initiative and concluded that manufacturers were on track to successfully phase-out microbeads in consumer products, and that the phase-out of microplastics in consumer products will be completed soon. At the initiation of the voluntary phase-out of plastic microbeads, their use in pharmaceutical dosage forms was not included.

Hazards to human health

While microplastics have been in use as ingredients in pharmaceutical dosage forms for more than 60 years, we do not yet fully understand how styrene-based microplastics interact with human tissue and their inherent risk level (Smith *et al* 2018). Therefore, manufacturers of pharmaceutical products, government regulatory bodies, health staff, and patients need to approach their use with an abundance of caution.

It has long been stated that microplastics in pharmaceutical dosage forms are safe because they are expected to be chemically inert and are not soluble, but there is now sufficient evidence that these claims are not correct. There is evidence that microplastics have the potential to affect human health in one of two ways; through direct damage to the lining of the gastrointestinal tract without their absorption, and through absorption/persorption that then causes damage in other regions of the body. The potential effects they have on the body can be from both physical and chemical pathways (Smith *et al* 2018).

Pharmaceutical dosage forms that contain microplastics are generally formulated as orally administered products designed to be chewed in the mouth (e.g. nicotine gum) or swallowed as either a suspension, solution, or solid tablet (Yoshida *et al* 2013). Some microplastic containing pharmaceutical products are formulated as eye drop solutions.

Even when not taken up from the gastrointestinal tract, styrene sulfonate-based microplastics can cause serious adverse effects. A recent review has highlighted 58 reported cases over a 63-year period where SPS microplastics have caused serious health problems (Harel *et al* 2013). Reported side effects to treatment with SPS included colonic necrosis, bowel wall ulceration and perforation, abdominal pain, distension, nausea and vomiting, gastrointestinal bleeding, and diarrhea. The review concluded that the colon is most affected by SPS microplastics, but that the small intestine can also be injured.

Importantly, there was a 33% mortality rate in patients who reported an adverse event to the microplastic (Harel *et al* 2013). Of the patients who died after being administered SPS microplastic treatment, 94% had colonic necrosis (Harel *et al* 2013). As well as localised adverse effects in the intestines, translocation of microplastics across the walls of the gastrointestinal tract is a now well documented phenomenon (Florence and Hussain 2001). There is also specific evidence in both human and animal models that this occurs for styrene sulfonate-based particles within the size range of 50 nanometres to 3 microns (Hoet *et al* 2004; Jani *et al* 1992).

Microplastic particles are hypothesised to be kneaded into the mucosal lining of the intestines during their passage through the gut. When this happens the microplastics that are of micron size are able to pass between the epithelial cells and into the subepithelial layer, where they are then transported around the body via the lymphatic system and the circulatory system (blood serum) (Vokheimer 1974). Translocation of microplastic into the body from the gut, and their eventual elimination, is evident by the detection of microplastics in the urine of some patients (Galloway 2015).

How much microplastic is taken up from the gastrointestinal tract is a function of the size and charge of the plastic (Hoet *et al* 2004), and the medical condition of the patient. Several studies have shown that styrene sulfonate-based microplastics are taken up differently in patients with diabetes (a medical condition linked to being overweight) (McMinn *et al* 1996). In animal models, rats with diabetes took up styrene sulfonate-based microplastic particles at a rate 100 times higher than the rate observed for normal rats (Hoet *et al* 2004).

Once in circulation within the body, microplastics can be deposited in various organs, including the liver (Jani *et al* 1992), in cerebrospinal fluid, in the peritoneal cavity, and in the alveoli of the lungs where they can remain for a considerable period of time (Haupt and Hutchins 1982). There is also evidence that particles can be excreted in breast milk and that they can cross the placenta (Vokheimer 1974).

There is direct evidence that SPS microplastics are aspirated in the lungs of patients after oral administration, and that this aspiration can lead to acute bronchitis and pneumonitis (Haupt and Hutchins 1982). Other effects that microplastics, including styrene sulfonate-based microplastics, can have on the human body include embolism of small vessels, inflammation, genotoxicity, oxidative stress, cellular apoptosis, and necrosis (Wright and Kelly 2017).

Of course, the effect that microplastics will have on the human body will be dependent on dose of the plastics and the length of the exposure (Hwang *et al* 2020). Acute and immediate effects are only likely when a person ingests a large quantity of microplastic particles, as would be the case in the treatment of hyperkalaemia. However recent research has shown that microplastics can accumulate in human tissue, as would happen from prolonged daily ingestion of medicines containing low doses of microplastics, and that the long-term chronic effects of this are likely to be negative for human health (Yong *et al* 2020).

Patients and informed consent

There are no medicines which are 100% safe and all medications have an element of risk and associated potential adverse effects. And because of these risks in health and medicine, it is expected that patients are provided an opportunity to give informed consent about their treatment. Patients grant medical personnel permission to administer their treatment, or provide a prescription, in full knowledge of the possible consequences (Cocanour 2017). The decision by a medical doctor to prescribe a medicine, and the acceptance by the patient to take the medicine, is based on their ability to weigh the perceived benefits against the risks. In order for a patient to exercise informed consent they need to have access to information that is both accurate and at a level that they can understand. Similarly, prescribing medical doctors and dispensing pharmacists need accurate and comprehensive information to be provided from the manufacturer or sponsor so they can pass this information on to their patients.

The first interaction a patient will have with a pharmaceutical product will be removing it from its packaging. It is therefore important that the packaging contains key information that the patient needs. Currently none of the products that contain styrene sulfonate state on their packaging that their product contains microplastic particles.

In Australia, the main two mechanisms for providing relevant information to health staff and patients are Product Information (PI) sheets and Consumer Medicine Information (CMI) sheets respectively. The PI documents are typically available via the internet on the ARTG. The CMI documents are also available via the internet but are also typically provided in a physical form with the relevant pharmaceutical product as well.

Therefore it is highly important that the information provided in PI and CMI documents for medicines containing microplastic is accurate and understandable, and that it does not leave out essential information. There is insufficient information provided in the CMIs for the three pharmaceutical products that contain styrene sulfonate microplastics on the ARTG. The CMI for Betoptic® S Eye Drops 0.25% only states in the ingredient list that it contains polystyrene sulfonate hydrogen but provides no explanation of this ingredient. Likewise, the CMI for Resonium A® states that it contains sodium polystyrene sulfonate, and that patients should tell their doctor or pharmacist if they have any allergies to "any other medicines which contain polystyrene sulfonate". The CMI does state that the Resonium does not enter the blood stream from the intestine and that it is passed with faeces. While technically correct in that the microplastic particles do not enter the blood stream from the gut, as they instead most likely transported via the lymphatic system, this statement can imply to patients that microplastics are not taken up at all by the body from the gut, and therefore the document downplays the potential risk.

Most importantly, neither of the two CMIs state that the SPS/HPS in the products are in the form of a microplastic. The CMI for Duromine™ does not list SPS as an ingredient in the document. Instead, it only states on the first page that the API (phentermine) is combined with a resin which slowly releases the drug. While it is common for the microplastics used in pharmaceutical dosage formulations to be referred as ion-exchange resins, or simply resins, it is unlikely that patients/consumers have a common understanding of what that means. The term resin can mean both: solid or semisolid natural organic substances that are secreted by plants and are soluble in organic solvents (such as ether) but not in water and any of a large class of synthetic products that have some of the physical properties of natural resins, but are chemically different and are used in the manufacture of plastics.

The former may bring to mind products such as tree sap and gum which are pliable and have flowing properties and are distinct from hard plastics. Resins may also be considered natural by consumers, in contrast to human-made plastics, which include SPS and HPS. As such, consumers taking a pharmaceutical product that contains an ion-exchange resin may not understand that this means they are ingesting microplastic particles. As it currently stands, the use of the term resin is unlikely to be understood by the average consumer and therefore does not provide them the opportunity to have informed consent on their treatment.

Prescribing doctors and dispensing pharmacists rely on PI documents to provide additional information beyond what is found in the CMI. This information includes significant chemical and clinical data and a full description of the expected adverse effects. In the PI documents for the three styrene sulfonate-containing pharmaceutical products listed on the ARTG, none of them sufficiently provide information on the potential harms of the microplastic ingredients. This means that health staff are not able to provide information to patients to allow them to give informed consent to their treatment.

Despite clear evidence that microplastics, and specifically styrene-based plastics, can be taken up into the body from

the gastrointestinal tract, the products all state that they are not taken up by the body. The PI for Resonium A® states that "sodium polystyrene sulfonate is not absorbed from the gastrointestinal tract" and does not mention that in numerous case studies SPS microplastics have been found aspirated in the lungs of patients (Haupt and Hutchins 1982). Neither the Betoptic® S or Duromine™ PIs provide any information on the pharmacokinetics or fate of the styrene sulfonate-based microplastics in the products. None of the PI documents state the styrene-based resins are in the form of a hard plastic.

Due to the lack of information on product packaging and provided documentation, patients are not in a position to provide proper informed consent for their use of pharmaceutical products that contain microplastics and use those medicines without understanding the risks.

Conclusion

Microplastics have been in use in various pharmaceutical dosage forms since the 1950s, and several are currently registered on the ARTG. The microplastics are used as ion-exchangers as either the API for the treatment of hyperkalaemia or as excipients for the purpose of providing sustained release of an API.

Plastics, microplastics, and microbeads pose a persistent threat to the environment. From sewage water these plastics enter the aquatic environment where they accumulate in both fresh- and salt- water systems, and in waterway sediments. They can then be ingested by a variety of animals where they can cause damage to aquatic life and also bioaccumulate in the food chain. Consumption of the seafood, such as shellfish and molluscs, then results in the re-ingestion of microplastic by people who would not have otherwise been exposed to microplastics that originate from pharmaceutical products. Recognising the severe environmental hazards that microplastics present, the Australian Government has introduced a voluntary phase-out of microplastics in the form of microbeads from consumer products. This phase-out has not yet been extended to pharmaceutical products.

Despite claims in the CMI and PI documents that microplastics, specifically styrene sulfonate-based ingredients, are not taken up by the body from the gastrointestinal tract and are therefore safe, there is considerable scientific and clinical evidence this is not the case. Without being taken up by the body, styrene sulfonate-based microplastics can have acute, serious adverse effects on the intestines, including colonic necrosis. When taken up into the body via absorption or persorption, the microplastics can be aspirated in the lungs which could lead to bronchitis, pneumonitis, embolism of small vessels, inflammation, genotoxicity, oxidative stress, and cellular apoptosis and necrosis. More research is needed to examine the fate of microplastic particles within the human body, and the effects that they can have on different organs and bodily systems.

Finally, the lack of clear language around microplastic ingredients means that patients are not afforded the opportunity to provide informed consent. Manufacturers therefore need to be compelled to provide clear statements on their products' packaging and information sheets that informs consumers and health care workers of the inclusion of microplastic particles within the medicines.

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Journal-based CPD No. 74
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Questions relating to the article *'A diagnostic menACE: the role of ACE-inhibitors in acute isolated hypotensive transfusion reactions and examination of the utility of tryptase levels*' at page 2 of this issue.

1.	The majority of AHyTR reported to be linked to a transfusion do not relate to the concurrent use of angiotensin-converting enzyme (ACE) inhibitor medication.	True/False
2.	The clinical symptom of hypotension following transfusion can be associated only with immunological causes.	True/False
3.	A large number of cases of isolated severe hypotension in the absence of other signs of inflammation, allergy, or haemorrhage have been previously documented.	True/False
4.	The utility of tryptase levels in these patients is then examined to determine the causes of tryptase elevation and the link between tryptase elevation and ACE inhibition.	True/False
5.	Tryptase levels on the samples collected at the time of both the first and second reaction were elevated at 20.6 µg/L and 19.2 µg/L (RR <15 µg/L) respectively.	True/False
6.	Laboratory investigation for red cell incompatible transfusion reaction excluded the presence of a haemolytic transfusion reaction and several sets of blood cultures remained sterile after five days.	True/False
7.	The report has not excluded platelet HPA incompatibility and did not recommend that this patient requires HLA matched platelets.	True/False
8.	Acute or immediate transfusion reactions occur within 24 h of the transfusion and often during the transfusion.	True/False
9.	Unlike plasma KKs, a continuous secretion of tissue KK can occur in organ-specific tissues, suggesting that tissue KKs contributes to physiological regulatory processes.	True/False
10.	Patients taking ACEi can suffer hypotension and angioedema.	True/False

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Journal-based CPD No. 75
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Questions relating to the article *'Microplastics in pharmaceutical dosage forms and patient informed consent'* at page 24 of this issue.

1.	Polystyrene sulfonate is a synthetic polymer that is used either as the active pharmaceutical ingredient or as a sustained release excipient in pharmaceutical products.	True/False
2.	There is not enough evidence that microplastics can be absorbed and persorbed from the gastrointestinal tract.	True/False
3.	Microplastic can also be aspirated in the lungs causing bronchitis and pneumonitis and can accumulate in organs such as the liver	True/False
4.	Many countries, including Australia, have approved specific microplastic-based medicines for the treatment of hyperkalaemia.	True/False
5.	Many API molecules have a sweet taste which reduces acceptability to patients and can lead to non-compliance with medications	True/False
6.	Microplastics are also being investigated for additional applications in pharmaceutical dosage forms.	True/False
7.	Using the UN Globally Harmonized System, styrene-based plastics have been assigned as a Class V hazard.	True/False
8.	The hazard rating for plastics is based on its chemical composition and the monomer from which it is manufactured.	True/False
9.	In 2018, the FDA also acknowledged the carcinogenic potential of styrene, and as a result, no longer permits its use as a food additive.	True/False
10.	Microplastics can turn up in the environment in one of two ways; from consumer goods and pharmaceutical products, and from the degradation of larger plastics.	True/False

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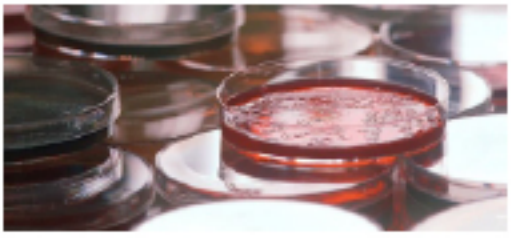

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1. **Bifidobacteria: Genomics & Molecular Aspects** edited by B. Mayo, & D. Van Sinderen. Caister Academic Press. xii + 260 pages.

2. **Medicine and Sport Science Volume 55: Cytokines, Growth Mediators & Physical Activity in Children during Puberty** edited by J. Jurimae, A.P. Hills & T. Jurimae. Karger. viii+178 pages.

3. **Digestive Diseases The Keys to IBD 2010: Treatment, Diagnosis & Pathophysiology.** Edited by G. Rogler & W. Sandborn. Karger. 188 pages.

4. **Else Kröner-Fresenuis Symposia Volume 1: Molecular Mechanisms of Adult Stem Cell Aging** edited by K.L. Rudolph. Karger. xii+108 pages**.**

5. **Endocrine Development Volume 24: Hormone Resistance and Hypersensitivity** edited by M. Maghnie, S. Loche, M. Cappa, L. Ghizzoni & R. Lorini. Karger. viii + 160 pages.

6. **Frontiers of Hormone Research Volume 41: Endocrine Tumor Syndromes and Their Genetics** edited by C.A Stratakis. Karger. xii + 187 pages.

7. **Frontiers of Hormone Research Volume 39: Kallmann Syndrome & Hypogonadotropic Hypogonadism** edited by R. Quinton. Karger. x+174 pages.

8. **Generic: The Unbranding of Modern Medicine** by Jeremy A. Greene. John Hopkins University Press. 368 pages.

9. **Human Pathogenic Fungi: Molecular Biology and Pathogenic Mechanisms** edited by Derek J. Sullivan & Gary P. Moran, Caister Academic Press. x + 342 pages.

10. **Internal Medicine: A Doctor's Stories** by Terrence Holt. Black Inc. 273 pages.

11. **Intolerant Bodies: A Short History of Autoimmunity** by Warwick Anderson and & Ian R. Mackay. John Hopkins University Press. 250 pages.

12. **More Than Hot: A Short History of Fever** by Christopher Hamlin. John Hopkins University Press. 400 pages.

13. **Pediatric and Adolescent Medicine Volume 19: Metabolic Syndrome and Obsesity in Childhood and Adolescence** edited by W. Kiess, M. Wabitsch, C. Maffeis, A.M. Sharma. Karger. x + 202 pages.

14. **Phage Therapy - Current Research and Applications** edited by Jan Borysowski, Ryszard Miedzybrodzki & Andrzej Gorski. Caister Academic Press. 368 pages.

15. **Shigella: Molecular and Cellular Biology** edited by William D. Picking & Wendy L. Picking. Caister Academic Press. 280 pages.

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The abstract should be approximately 150 words and should make sense when read alone or in conjunction with the article. The abstract should be a concise overview that describes the important details of the article including the purpose of the study/investigation, basic procedures (study subjects/experimental animals/observational and analytic methods) and the results and principal conclusions. New and important aspects of the work and its implications may also be included. References should not be included.

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Acknowledgements

Acknowledge individuals who have made substantial contributions to the study including technical work and financial support. Authors are responsible for obtaining consent from all the individuals acknowledged by name as inclusion may be interpreted as an endorsement of the article's contents.

References

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Osler AG 1976. *Complement: mechanisms and functions*. Englewood Cliffs: Prentice-Hall.

Editor, Compiler, Chairman as Author:

Rhodes AJ, Van Rooyen CE, comps. 1968. *Textbook of virology: for students and practitioners of medicine and the other health sciences*. 5th ed. Baltimore: Williams and Wilkins.

Chapter in Book:

Weinstein L, Swartz MM 1974. Pathogenic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, eds. *Pathologic physiology: mechanisms of disease*. Philadelphia: WB Saunders; 457-472.

Online documents:

National Center for Biotechnology Information. OMIM: online Mendelian inheritance in man. http://www.ncbi.nlm.nih.gov/omim. Accessed February 25, 2007.

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g	gravity
Hz	hertz
h	hour
IU	international unit
K	kelvin
kg	kilogram
L	liter, litre
m	meter, metre
min	min
M	molar
mL	millilitre
mol	mole
N	newton
nm	nanometre
p	probability
rpm	revolutions per min
s	second
wk	week
yr	year

Additional information

The following are useful sources of information. The first two publications are used by the AJMS as standard references.

Style Manual Committee. Council of Biology Editors. *Scientific style and format: the CBE manual for authors, editors, and publishers.* 6th ed. Cambridge University Press, 1994.

Style manual for authors, editors and printers. 6th ed. John Wiley & Sons Australia Ltd, 2002.

O'Connor M, Woodford FP. *Writing scientific papers in English: an ELSE-Ciba Foundation guide for authors.* Amsterdam, Oxford, New York: Elsevier-Excerpta Medica, 1975.

Day RA. *How to write and publish a scientific paper.* Philadelphia, Institute for Scientific Information Press, 1979.

Zeiger M. *Essentials of writing biomedical research papers*. 2nd ed. New York, McGraw-Hill, 2000.

Matthews JR, Matthews RW. *Successful scientific writing: a step-by-step guide for the biological and medical sciences*. 3rd ed. Cambridge, Cambridge University Press, 2007 [Also available in eBook format.]

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