

Ant venom immunotherapy: a double-blind, placebo-controlled, crossover trial

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Summary

Background The jack jumper ant *Myrmecia pilosula* is responsible for about 90% of ant venom anaphylaxis in southeastern Australia. We aimed to establish whether *M pilosula* venom immunotherapy (VIT) prevents lifethreatening sting anaphylaxis in otherwise healthy adults.

Methods We did a double-blind, placebo-controlled crossover trial of *M pilosula* VIT. Participants were randomly allocated either immunotherapy, in accordance with the semirush hyposensitisation regimen, or placebo. The primary endpoint was systemic reaction after a deliberate sting challenge. Analysis was per protocol.

Findings We randomly allocated 68 healthy volunteers (aged 20–63 years) who were allergic to *M pilosula* venom to placebo (33) and VIT (35). Four on placebo were stopped early and 12 on VIT had their treatment allocations revealed before the sting challenge, thus 29 on placebo and 23 on VIT were included in the primary analysis. Objectively defined systemic reactions to sting challenges arose in 21 of 29 participants (72%) on placebo (8 reactions were associated with hypotension) and none of 23 on VIT ($p < 0.0001$). Of the remaining 12 on VIT who underwent sting challenges after treatment allocations were revealed, only one reacted to sting challenge with transient urticaria that did not require treatment. After crossover of the placebo group to VIT, one of 26 had a reaction to sting challenge (transient urticaria). In all patients who had VIT, we recorded objective systemic reactions in 22 of 64 (34%) during VIT; two of which were hypotensive.

Interpretation In well motivated, highly allergic, but otherwise healthy adults, VIT is highly effective in prevention of *M pilosula* sting anaphylaxis. The risk of systemic reactions during VIT means that treatment should be given where there is immediate access to resuscitation facilities.

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Introduction

Most native Australian stinging ants belong to the genus *Myrmecia* (bull ants), which includes 89 recognised species.¹ Results from serological and clinical studies implicate the aggressive species complex *M pilosula* (jack jumpers, figure 1) in about 90% of allergic reactions to ant venom in southeastern Australia.^{2–4} In Tasmania, the rate of clinical systemic allergy to *M pilosula* venom is 2.7% (95% CI 1.8–3.9%), compared with 1.4% for bee stings,³ and deaths have been reported.⁵

For the treatment of bee and wasp sting allergy, venom immunotherapy (VIT) is thought to be an effective treatment, whereas whole-body extract (WBE) seems to be no better than placebo.⁶ The evidence base for venom immunotherapy consists of results from: (1) a single-blind trial comparing VIT, WBE, and placebo with non-random allocation to treatment groups on the basis of factors now known not to affect reaction risk;⁶ (2) a small open-label trial comparing VIT with WBE, in which accidental field stings were the primary endpoint, and in which neither the causative insects nor patients' reactions were seen by the investigators;⁷ and (3) uncontrolled observational studies reporting failure rates of between 0% and 9% for wasp VIT and between 0% and 42% for bee VIT.⁸

So far, treatments for allergy to ant sting have used only WBE. Fire ant (*Solenopsis* spp) WBE is used in the USA; its effectiveness is lent support by promising case series, but randomised controlled studies have not been done.^{9,10} In Australia, *M pilosula* WBE was withdrawn in the early 1990s and reported, on the basis of a retrospective analysis of field stings to be ineffective.¹¹ Our aim was to assess the effectiveness of *M pilosula* VIT in prevention of life-threatening sting anaphylaxis.



Figure 1: Jack jumper ant, *Myrmecia pilosula*

Seen here depositing venom on a glass slide in response to electrical stimulation. This ant is 10–12 mm long and jet black except for yellow-orange mandibles and leg tips. It jumps to attack, grasps firmly with its mandibles, and injects venom with an abdominal stinger.

Grading of systemic allergic reactions

I	Generalised urticaria* or erythema, itching, malaise, or anxiety
II	Angioedema* or two or more of: chest or throat tightness; nausea, vomiting, diarrhoea, abdominal pain, or dizziness
III	Dyspnoea, wheezing, or stridor or two or more of: dysphagia, dysarthria, hoarseness, weakness, confusion, or feeling of impending disaster
IV	Hypotension, collapse, loss of consciousness, incontinence of urine or faeces, or cyanosis

*Periorbital oedema was judged a manifestation of urticaria, not angioedema. Grading system after U Muller.⁸

Methods

Participants

We recruited healthy volunteers who: were aged between 17 and 65 years; had a history of grade II–IV hypersensitivity to a clearly identified or presumed *M pilosula* sting (panel); did not have a history of hypertension, heart disease, poorly controlled lung disease, ACE-inhibitor or β -blocker therapy; were willing to undergo a sting challenge with the risk of severe, potentially life-threatening anaphylaxis; and had a positive skin test, defined by wheal growth of at least 2 mm and flare greater than or equal to 10 mm in response to an intradermal injection of *M pilosula* venom (about 0.02 mL to form a 3–5 mm bleb) at a concentration of 1.0 mg/L or less. If results of the skin test were negative, we repeated the test before declaring a negative result. People with negative skin tests, although excluded from this study, were advised of the potential risk from future stings and were provided with appropriate action plans. We measured tryptase concentration in blood using a fluoroenzyme immunoassay (Unicap Tryptase, Pharmacia and Upjohn, normal range <12 μ g/L) and serum venom-specific IgE with radio-allergen-sorbent-test (RAST) as previously described.² Participants gave written informed consent, and were given clear written and verbal explanation of the potential risks of immunotherapy and live-insect sting challenge. The Royal Hobart Hospital and Flinders Medical Centre ethics committees approved the study protocol.

Procedures

An investigator not involved in direct patient care used a random number-based sequential allocation process to allocate participants to treatment groups. We dispensed patient-specific treatment packs that contained either lyophilised venom or placebo and a diluent solution of 0.05% polysorbate 80. Packs and contents were identical in appearance. We did a double-blind placebo-controlled trial to assess efficacy of VIT in prevention of sting anaphylaxis. We used a deliberate sting challenge done in the emergency departments of two Tasmanian public hospitals, the Royal Hobart Hospital and North West Regional Hospital, Burnie.

Venom was obtained by dissecting venom sacs of *M pilosula* gathered at various locations around Tasmania. Venom was pooled into a large batch, lyophilised, weighed, and then dispensed into vials containing 50 or 120 μ g of venom protein and 20 mg mannitol. We lyophilised the preparations again, which were then vacuum-sealed and stored below 8°C. In placebo vials, 100 μ g venom protein was substituted with 7 μ g or 8.4 μ g histamine acid phosphate to simulate the anticipated variability in local reactions.

Visit	Amount of venom (μ g)		
1	0.0001	0.001	0.01
2	0.01	0.1	0.3
3	0.3	1	3
4	3	10	..
5	10	20	..
6	20	30	..
7	30	40	..
8	50	50	..
9	70	30	..
Maintenance	100	..	..

Injections were given at 20–60 min intervals. This regimen was used as a guide only, and adjusted in accordance with patient tolerance. Visits 1–10 were 5–10 days apart, then gradually extended to monthly maintenance visits. The minimum period of observation after the last injection of each visit was 1 h.

Table 1: Outpatient semi-rush hyposensitisation regimen

We gave VIT in accordance with an outpatient semirush procedure (table 1), choosing an arbitrary maintenance dose of 100 μ g, on the basis of doses used in bee and wasp VIT. The first dose was determined by the threshold at which patients were positive to intradermal skin testing—ie, 0.1 mL of a solution at a tenth of the concentration that produced a positive skin test. For example, 0.00001 μ g for participants who had a positive skin test at 0.001 mg/L. This regimen was used as a guide only, and was adjusted in accordance with patient tolerance. Participants were observed for at least 1 h after the last dose was given. We allowed patients who had adverse reactions to take prophylactic oral antihistamines (10 mg loratadine with 300 mg ranitidine taken 2 h before immunotherapy).

We graded immediate-type hypersensitivity reactions to immunotherapy using the categories outlined in the panel but with the following modifications: at least one objective physical sign was needed to define a systemic reaction of any grade; and grade IV reactions were assigned only when systolic blood pressure was less than 90 mm Hg.

Patients received sting challenges in an emergency department resuscitation room, 1 week after tolerating two successive VIT maintenance doses. Antihistamines were not taken for at least 1 week before the challenge. We monitored patients using electrocardiography, non-invasive blood pressure measurements, and continuous pulse-oximetry. With a 10 mL syringe device,¹² we pushed a single jack jumper ant against the ventral forearm and allowed the ant to sting for 60 s. If no objective physical signs of a systemic reaction were evident within 30 min, we used a fresh ant to give a second sting. We gave this second sting because during venom sac dissection we noted that one in five venom sacs was empty. We took blood samples from patients at baseline, 15 min, and 60 min after the final sting. Histamine values were measured by ELISA (IBL, Hamburg, Germany, normal range <1.0 μ g/L) on samples taken at baseline and at 15 min. Tryptase concentrations were measured in samples from baseline, 15 min, and 60 min.

Unless the trial participant wanted symptoms to be alleviated, we allowed reactions to progress until they were of stable severity for 30 min, or until peak expiratory flow rate fell to less than 60% of that at baseline, pulse oximetry saturation was under 92% breathing air, systolic blood pressure was less than 90 mm Hg, or if there was evidence of myocardial ischaemia. We graded reactions in the same way as we did hypersensitivity reactions to VIT. We gave intravenous epinephrine starting at 5–17 μ g/min, then adjusted the dose in accordance with

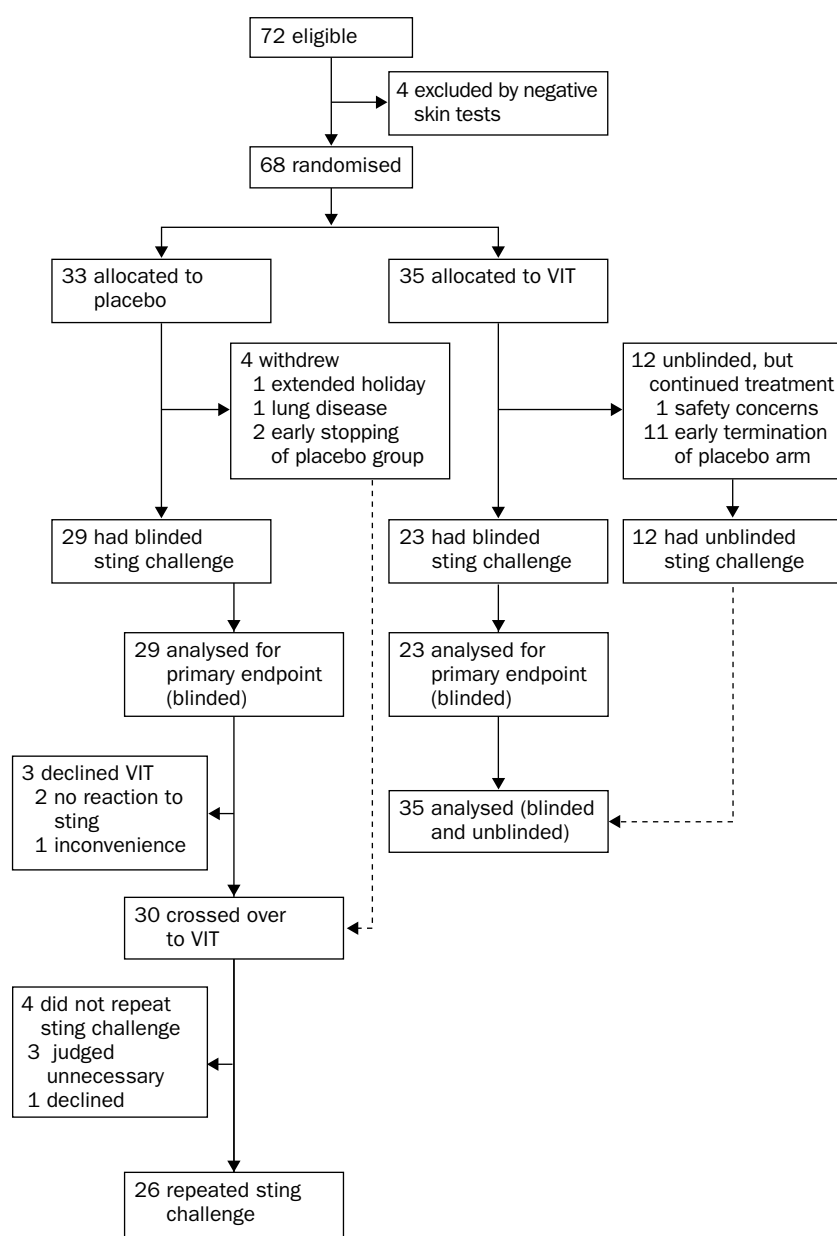


Figure 2: Trial profile

clinical response, and gave other resuscitation as needed. We allowed patients to leave hospital if they had been free of symptoms for at least 2 h after epinephrine infusion had been stopped, or 1 h after sting challenge if there had not been a reaction. To avoid continued ineffective treatment after primary endpoints had been measured, patients and investigators were told whether the patient had been given placebo or active treatment after the result of the sting challenge was known and structured datasheets had been completed.

Participants in the placebo group were offered VIT after the first part of the trial had been completed. The sting challenge was repeated after immunotherapy unless the patient had not reacted to the first sting challenge given after placebo.

Statistical analysis

Our power analysis showed that 40 people in each study group would give 85% power to detect a difference with a significance of $\alpha=0.05$ with a two-tailed test, and

assuming treatment failure rates of 30% (placebo) and 5% (active treatment), because these were the average in-hospital sting challenge reaction rates in untreated individuals with a history of bee or wasp venom allergy and the average reported failure rate of VIT, respectively.^{8,13}

The primary outcome measure was a systemic reaction to sting challenge defined by objective measurements. Secondary outcome measures were any systemic symptoms in the absence of objective physical signs, a grade IV reaction, treatment with epinephrine, and changes in serum mast cell tryptase or plasma histamine after the sting challenge. We planned to do an interim analysis after the first 30 participants had completed the study. Fisher's exact and χ^2 tests were used to compare dichotomous and ordinal variables. We used the Mann-Whitney *U* test for continuous baseline variables, which had non-normal distributions. The median test was used to compare continuous outcome variables, all of which had non-normal distributions that differed in shape between groups.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. NSL Health Limited (Melbourne, Australia) discontinued allergy research activities and relinquished its commercial interests during the trial.

Results

Between August 6, 2001, and Oct 4, 2001, 72 people were judged eligible for the trial on the basis of reaction histories, physical health, and willingness to undergo a sting challenge. We excluded four because of negative skin tests—these people also had undetectable or equivocal serum IgE reactivity and did not have positive skin or serum IgE reactivity to bee, wasp, or venom from any other local *Myrmecia* spp. Thus, 68 individuals were randomly allocated to one of the two study groups—33 to placebo and 35 to VIT (figure 2). Table 2 shows participants' baseline characteristics. Table 3 shows main results of VIT, including adverse reactions.

After the first 30 participants completed sting challenges, a substantial imbalance between numbers in placebo and VIT groups was apparent (19 *vs* 11, respectively). Interpretation of results from these 30 participants was difficult because taking a long time to achieve tolerance of the maintenance dose could well have been the result of treatment failure, and an interim analysis that excluded such cases would have been seriously biased. Therefore, we continued the trial.

In the placebo group, one participant departed overseas. Another withdrew because of newly diagnosed

	Placebo (n=29)	VIT (n=35)
Age (years)	46 (20–62)	50 (18–63)
Men	18 (62%)	22 (63%)
Asthma	0	5 (14%)
<i>M pilosula</i> clearly identified as cause of previous systemic reaction	29 (100%)	34 (97%)
Worst previous reaction grade		
Grade II	1 (3%)	5 (14%)
Grade III	20 (69%)	16 (46%)
Grade IV	8 (28%)	14 (40%)
Documented hypotension with sting	2 (7%)	9 (26%)
Previous treatment with epinephrine	17 (59%)	29 (83%)
Age at first systemic reaction (years)	35 (4–60)	40 (9–49)
Time since last sting (years)	3 (0–23)	2 (0–35)
Number of sting reactions	2 (1–20)	2 (1–24)
Intradermal skin test positive threshold		
1.0 mg/L	7 (24%)	5 (14%)
0.1 mg/L	11 (38%)	14 (40%)
0.01 mg/L or less	11 (38%)	16 (46%)
Venom-specific IgE *	1.9 (0.3–19)	3.8 (0.2–32.8)
Raised baseline mast cell tryptase	0	3 (9%)†

Data are number (%) or median (range). *Venom specific serum IgE expressed as percentage uptake of radioactive tracer. †Tryptase concentrations (normal range <12 µg/L) were 16.3 µg/L, 18.7 µg/L, and 23.2 µg/L; all had a history of grade IV anaphylaxis, and one had historical features and skin findings suggestive of mastocytosis (telangiectasia macularis eruptiva perstans).

Table 2: **Baseline characteristics of participants who underwent sting challenge**

airways disease with deteriorating exercise tolerance. In the VIT group, one person developed debilitating panic attacks with flashbacks to a previous near-lethal reaction. We decided that concealment of the patient's treatment was a contributing factor to their anxiety. After treatment allocation was revealed and psychiatric treatment was started, the panic attacks resolved and the patient continued with VIT.

After 52 sting challenges to participants for whom treatment allocation was not known, a severe grade IV reaction prompted us to attempt another interim analysis. Of these challenges, objective reactions were noted in 21 of 29 (72%) in the placebo group and none of 23 in the VIT group ($p<0.0001$). We calculated that the differences in the reaction rates would remain significant even if the remaining two participants in the placebo group did not react and the remaining 12 participants in the VIT group did react to sting challenge ($p=0.0130$). Therefore, we ceased treatment in the placebo group, and all remaining treatment allocations were revealed.

In the placebo group, 15 anaphylactic reactions arose after the first sting and six after the second sting. Table 3 shows the severity of reactions to sting challenges. No participant requested treatment before the onset of severe reactions that were our endpoints. All responded well to treatment and made a full recovery. Two participants with severe reactions had severe bradycardia. One, a 41-year-old man with a history of grade III reactions, remained hypotensive for 15 min despite an epinephrine infusion at 32 µg/min and rapid volume expansion with 3 L of normal saline. After initial tachycardia, he became progressively bradycardic, but responded to atropine. The other, a 57-year-old woman with a history of grade IV reactions, suddenly became unconscious with agonal respirations, became erythematous, had no palpable pulses, and had a bradycardia of 37 beats per min. She responded to simultaneous treatment with atropine, epinephrine, and rapid infusion of 1 L of normal saline.

The severity of reaction to the deliberate sting in the placebo group did not seem to be predicted by the previous worst grade reaction to an accidental sting

	Placebo* (n=32)	VIT (n=35)	p
Immunotherapy			
Number of visits†	11 (9–18)	15 (9–35)	0.003
Cumulative dose (µg)‡	568 (431–706)	648 (466–1971)	<0.001
Histamine premedication	2 (6%)	17 (49%)	0.0002
Minor systemic symptoms	15 (47%)	27 (77%)	0.014
Number of people experiencing objective systemic reactions	1 (3%)	13 (37%)	0.0010
Treated with epinephrine	1 (3%)	7 (20%)	0.0064
Unable to reach 100 µg target dose	0	2 (6%)	0.5548
Sting challenge			
Any subjective symptoms‡	26 (90%)	13 (37%)§	<0.0001
Systemic reactions	21 (72%)	1 (3%)§	<0.0001
Grade I	7	1 (3%)§	
Grade II	3	0	
Grade III	3	0	
Grade IV	8	0	0.0019
Treated with epinephrine	19 (66%)	0	<0.0001
Change tryptase (µg/L)	1.4 (–3.7 to 31.3)	0.0 (–2.6 to 3.6)	<0.001
Change histamine (µg/L)	0.98 (–2.1 to 164)	0.0 (–4.1 to 3.5)	0.0287

Data are number (%) or median (range). *Three completed placebo immunotherapy, but withdrew before sting challenge. †Median (range) number of visits and cumulative dose were calculated up to and including the second maintenance dose. In people receiving VIT, seven had 20 or more visits and received 1000–1971 µg of venom each. In four people, there were further delays to sting challenge ranging from 5–19 weeks, because of difficulties in toleration of the 100 µg maintenance dose. ‡Including tingling, transient itch, facial warmth, palpitations, and headache. §Before early stopping of placebo and unmasking of treatment allocations, the rate of subjective symptoms to sting challenge in the VIT group was five of 23 and objective reaction features none of 23.

Table 3: **Outcomes of randomised study phase**

(table 4), shown by five grade IV reactions that arose in the 20 people who had a history of grade III reactions.

After completion of sting challenges in the remainder of the VIT group, only one patient had a systemic reaction: a small patch of urticaria that resolved without treatment. This person had had a hypotensive reaction and needed resuscitation when last stung before trial entry. Maintenance doses of 100 µg had been tolerated without any side-effects before the sting challenge.

30 participants from the placebo group chose to receive VIT. Sting challenges were not repeated in three who did not have a reaction to sting challenge after placebo treatment and in one who had not completed placebo treatment and declined a sting challenge. Of the remaining 26, only one reacted to sting challenge after crossover to VIT; this was a mild grade I urticarial reaction after one sting, that settled without treatment in a person who had a history of a grade IV sting reactions before trial entry, and who was unable to tolerate a maintenance dose greater than 50 µg. Table 5 shows adverse reactions to VIT, including those in the crossover group.

	Sting challenge reaction grade					Total
	No reaction	I	II	III	IV	
Worst reaction to accidental sting before trial						
Grade II	1					1
Grade III	5	4	3	3	5	20
Grade IV	2	3	0	0	3	8

Spearman rank correlation $p=0.99$.

Table 4: **Sting challenge reaction grade and previous worst reaction grade in the placebo group**

	Number affected (%) (n=65)	95% CI
Minor systemic symptoms*	46 (71%)	58.2–81.4
Immediate-type hypersensitivity reactions†	22 (34%)	22.6–46.6
Worst grade I	16	
Worst grade II	1	
Worst grade III	3	
Worst grade IV	2	
Treated with epinephrine	8 (12%)	5.5–22.8
Unable to achieve maintenance because of repeated systemic reactions‡	3 (5%)	1.0–12.9

*Included generalised itch, vagueness, tingling of the lips or limbs, facial heat, tiredness or lethargy, chest pain, nausea, headache, abdominal pain, sensation of puffy eyes, perspiration, and abnormal taste in the mouth. †All three participants with raised baseline tryptase concentrations had hypersensitivity reactions during immunotherapy. ‡Tolerated maintenance doses were 50–80 µg, despite antihistamine premedication. Only one of these three patients reacted (mild grade I, compared with previous grade IV reactions) to sting challenge.

Table 5: Adverse reactions to VIT from randomised and crossover phases

Discussion

This double-blind randomised trial of VIT has shown that VIT is an effective treatment for allergy to insect venom. However, how VIT could benefit the small proportion of skin-test negative patients is still not clear. In our study, four excluded people clearly reacted to *M pilosula* but were skin test negative, and had had obvious sting reactions, with no skin or serum IgE reactivity to any local insect species. This dilemma also applies to other hymenoptera sting allergies.¹⁴

We thought a placebo group was an essential feature of the study for several reasons. First, because failure to rigorously determine treatment efficacy in prevention of severe reactions could lead to a false sense of security for patients, a contributing factor to the death of a man who erroneously believed that he was protected by WBE immunotherapy.⁵ Second, we did not know how effective our *M pilosula* sting challenge procedure would be in causing severe anaphylaxis, and thus how accurately we could assess VIT efficacy. Furthermore, the natural history of sting allergy is unpredictable, and many people lose their sensitivity over time,^{6,13,15} and finally because no other treatment was available for comparison. Double-blinding was necessary because many sting reaction features could be open to subjective interpretation and because patient anxiety might have affected the results.

Other investigators^{13,16} who have done in-hospital sting challenges in patients allergic to bee and wasp venom showed that the procedure would be safe if patients with known cardiovascular disease were excluded, and even if severe reactions were allowed to resolve without the use of epinephrine.¹⁷ Although workers in one small series reported several serious reactions to sting challenge,¹⁸ no health or age-based exclusion criteria seem to have been applied, and participants had been exposed to large test doses of venom antigen immediately before the sting challenge, which probably contributed to the severity of reactions.

To adequately assess the efficacy of immunotherapy, we intended to leave sting reactions untreated until the patient reached objectively defined stages of respiratory or cardiovascular compromise. Because cardiorespiratory compromise, which usually arises rapidly after the sting,^{13,17} is the generally accepted threshold for resuscitation with epinephrine¹⁹ we did not judge this approach to carry substantial additional risk, especially because it was done in a critical-care environment.

Randomisation was effective, and groups were evenly matched in terms of characteristics known to be predictive

of reaction severity—namely, age and severity of worst previous reaction.^{3,13} With respect to blinding, clinically significant side-effects that distinguished people receiving VIT were unavoidable. Nevertheless, half the participants in the placebo group had systemic symptoms and many in the VIT group had few problems (table 3) and progressed rapidly to maintenance dose early in the study before the placebo group was stopped. These results indicate that study blinding was sufficient to maintain objectivity.

Untreated participants had a substantial risk of a severe reaction, with a reaction rate similar to the 70% (95% CI 61–78) seen after accidental field stings in people allergic to *M pilosula* venom,³ suggesting that the results of our in-hospital sting challenges will be predictive of reactions to accidental stings. These reaction rates are much higher than the 27–57% rates reported in prospective studies of untreated people with allergy to bee and wasp sting.^{6,13,16,20–23} We do not know whether this high rate is because *M pilosula* venom is an unusually potent allergen, because of high yearly exposure rates in Tasmania that might maintain sensitivity in allergic patients,³ or the ability of *M pilosula* to reliably deliver venom by virtue of its strong grasp and sting mechanism (figure 1). However, the clinical relevance of our findings is clear; the use of a diagnostic sting challenge to determine suitability for immunotherapy, as suggested for bee or wasp venom allergy¹³ is unnecessary, indeed dangerous, in those with a clear history of severe allergy to *M pilosula* venom and a positive skin test.

It is noteworthy that several people with a history of grade III reactions had grade IV reactions after the sting challenge. Although not identified in one series of sting challenges,¹³ progression of reaction severity has been documented previously for *M pilosula*,³ as well as for bee and wasp sting allergy.¹⁶ Outside a critical care environment, hypotension might not be detected because it can be a transient event associated with largely subjective symptoms.¹⁷ Thus, some reactions will probably be graded as more severe when closely monitored. Also, since severity of sting reactions is known to fluctuate over time, initial reactions might be minor and subsequent reactions more severe.

As with VIT for other sting allergies, the risk of systemic reactions to *M pilosula* venom means that treatment should be given where there is immediate access to resuscitation facilities. Hypersensitivity reactions during VIT occurred in all three patients who had raised baseline concentrations of tryptase, which lends support to mast cell disease as an important factor in some people with severe allergy.²⁴ The proportion of participants who had hypersensitivity reactions and failed to achieve the target maintenance dose were much the same as those reported for outpatient bee VIT, but greater than for wasp VIT.²⁵ Older people and those who have important comorbidities might have more severe reactions than would others. Real-life application of VIT in less motivated patients than those in this study might also result in poor outcomes if patients withdraw because of adverse effects of the treatment.²⁵ In this context, the minimum effective dose in *M pilosula* VIT remains to be defined.

In our highly motivated, highly allergic, but otherwise healthy study population who had positive intradermal skin tests, VIT was very effective in prevention of life-threatening sting anaphylaxis. VIT has some potential to prevent deaths and can result in striking improvements in the quality of life of affected individuals.²⁶ Ant VIT could benefit about 1% of the population (those with severe allergy) in areas of southeastern Australia where *Myrmecia* ant stings occur,^{3,4} and should be offered to those with skin test venom reactivity and a history of grade III–IV reactions.

Contributors

S Brown and M Wiese were responsible for ant collection and identification, venom extract formulation, and quality control. M Wiese did randomisation and dispensed treatment packs. S Brown and R Heddle were the main contributors to clinical trial design. R Heddle provided initial training in skin testing and the delivery of immunotherapy, and clinical support during the trial. S Brown and K Blackman supervised immunotherapy and did sting challenges. All investigators contributed to the writing of the report.

Conflict of interest statement

None declared.

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