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RESEARCH LETTER

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# Venom immunotherapy: safety and tolerability of the build-up phase with depot versus aqueous preparations

To the Editor,

Insect stings can cause an allergic reaction ranging from large local reaction (LLR) to systemic sting reactions (SSRs). Venom immunotherapy (VIT) is the only effective treatment in preventing systemic reactions at the subsequent field sting. VIT includes the so-called build-up phase and the maintenance phase, according to European and Italian guidelines.[1,2] An EAACI multicentre study revealed an incidence of 20% side-effects to VIT, the majority of which occurred in the build-up phase.[3] In another European multicentre study, the risk of systemic reaction during build-up phase was 8.4%.[4] In Europe, available Hymenoptera venom preparations used to perform VIT are divided into aqueous and depot preparations, which can be purified or not.[1] In most European countries, VIT is still performed with aqueous preparations, especially during VIT induction phase with different protocols (slower or faster), while the maintenance phase can be pursued with depot preparations.[5] Depot preparations are generally associated with fewer local side-effects than aqueous preparations during VIT maintenance phase. The slower build-up phase with depot preparations and the slow release from the injection site of allergen adsorbed on to aluminium hydroxide or L-tyrosine are considered as an advantage.[5,6] At the moment only few studies, based on a limited population size, provide definite evidence on build-up phase side-effects using depot versus aqueous venom preparations. Instead, there is little doubt about the efficacy and the lower side-effect rate using depot preparations during VIT maintenance phase.[1,5] The aim of this study is to retrospectively evaluate the safety of VIT build-up phase comparing commercially available purified depot and aqueous venom preparations.

Between January 2010 and July 2020, patients with a clinical history of hypersensitivity to Hymenoptera venom, consecutively administered VIT according to EAACI Guidelines and Italian Consensus criteria,[1,2] were retrospectively enrolled in the study as notified to the Ethical Committee. After appropriate informed consent, patients decided to be admitted to a 3-week protocol with purified aqueous preparation or to a 6-week protocol with purified depot preparation, depending on personal choice. During the build-up phase, subcutaneous injections were performed at incremental doses until the maintenance dose of 100 μg was reached. Protocols adopted in the build-up phase were a 6-week cluster/rush modified induction schedule[7] for depot preparations, or a cluster protocol in 3 weeks by Tahrini[8] (slightly modified in the doses of the second day, reaching a cumulative dose of 70 μg instead of 90 μg) for aqueous preparations (additional information is available in the following repository: https://doi.org/10.5281/zenodo.6831578). No antihistamine or Omalizumab premedication were used, as the first could mask a mild reaction, while the second is considered off-label in Italy during VIT. Venom purified depot preparations were: Alutard SQ adsorbed onto aluminium hydroxide (ALK-Abellò) and L-tyrosine-adsorbed preparation (Anallergo). Venom purified aqueous preparations were: Aquagen SQ (ALK-Abellò) and Anallergo (Florence, Italy). Systemic and local side-effects were recorded in the outpatient regimen procedure. LLRs and SRs were treated, depending on the severity. When no reactions occurred, the observation time after the last administered dose was 3 h. All patients were instructed to report any delayed reactions to the Allergy Unit, and they were interviewed during the next visit about any reaction or discomfort occurred within 24 h after VIT. Data were stored in a Microsoft Access database. We compared two groups (patients receiving build-up phase with depot vs. aqueous preparations) using chi-squared or Wilcoxon rank-sum test, for categorical and quantitative variables respectively. We fitted multivariable Poisson regression models with robust variance to calculate risk ratios (RR) of adverse reactions and 95% confidence intervals (CI) according to preparation type (depot vs. aqueous) adjusted for selected potential confounders, including gender, age class, venom type and Mueller grade. In a sensitivity analysis, we fitted a model using continuous age (in decades). Analyses were performed with Stata 17 (StataCorp. 2021).

We consecutively enrolled 444 adult venom allergic patients (age range 18–86 years). Among these, we excluded: three patients treated with two venom preparations (wasp and honeybee), eight patients suffering from systemic mastocytosis (due to likely higher reaction risk) and 15 patients who underwent a rush induction protocol (as faster protocols may be associated with more frequent adverse events).[1] Statistical analyses were performed on 418 patients: 258 (61.7%) and 160 (38.3%) were respectively treated with purified depot or purified aqueous preparations (Table 1). Among patients submitted to VIT with depot formulation, there were more males than females (70.1% vs. 29.9%). A similar

distribution was observed among patients treated with aqueous preparations (75.6% vs. 24.4%). Patients treated with depot preparations were younger (average 50.1 years, range: 18.7–78.3) than those with aqueous preparations (54.5 years, range: 18.0–85.9; $p = .005$). Hymenoptera venom types were equally distributed: wasp VIT was the most frequently performed both in patients treated with depot (87.2%, 225 of 258) and aqueous preparations (88.8%, 142 of 160; $p = .64$); honeybee VIT was less frequently performed both in patients treated with depot (12.8%, 33/258) and aqueous preparations (11.2%, 18/160). The overall cumulative incidence of reactions during induction phase was 9.1% (38/418; 95% CI: 6.7–12.3%). In patients treated with depot preparations 5 LLRs and 7 SRs occurred, while among those treated with aqueous preparations 20 LLRs and 6 SRs occurred. The risk of SRs was similar in both populations: 2.7% with depot preparations and 3.7% with aqueous preparations ($p = .55$). Considering LLR, females had a slightly elevated risk compared to males (Table 2). LLR occurred only among subjects aged 40 years or more. The multivariable Poisson model with continuous age yielded a RR of 1.27 (95% CI: 1.01–1.60), that is, a 27% increase per decade of age (95% CI: 1–60%). The Mueller grade pre-VIT had low influence on LLR during the build-up phase. Patients treated with honeybee venom had a slightly reduced adjusted risk, but with a wide CI. The relative risk of LLR was much lower (RR 0.16, 95% CI: 0.06–0.41, $p < .001$) in those treated with depot preparations (1.9%) compared to those treated with aqueous preparations (12.5%). These results were confirmed by multivariable analyses, in which patients treated with depot preparations had an adjusted RR of 0.15 (i.e., 85% decreased risk of LLR during build-up phase, $p < .001$); RR and CIs were identical when we fitted the multivariable Poisson model with continuous age.

**TABLE 1** Demographic data and clinical characteristics of the 418 patients under investigation

| | Depot | | Aqueous | | |
|---|---|---|---|---|---|
| | *N* | % | *N* | % | *p*-Value* |
| All | 258 | 100 | 160 | 100 | |
| Gender | | | | | |
| Males | 172 | 66.7 | 121 | 75.6 | .05 |
| Females | 86 | 33.3 | 39 | 24.4 | |
| Age (years) | | | | | |
| 18–39 | 68 | 26.4 | 27 | 16.9 | .15 |
| 40–49 | 60 | 23.2 | 35 | 21.9 | |
| 50–59 | 59 | 22.9 | 40 | 25.0 | |
| 60–69 | 48 | 18.6 | 38 | 23.7 | |
| 70+ | 23 | 8.9 | 20 | 12.5 | |
| Mueller grade pre-VIT | | | | | |
| I | 43 | 16.7 | 16 | 10.0 | .02 |
| II | 41 | 15.9 | 23 | 14.4 | |
| III | 73 | 28.3 | 34 | 21.2 | |
| IV | 101 | 39.1 | 87 | 54.4 | |
| Venom type | | | | | |
| Wasp | 225 | 87.2 | 142 | 88.8 | .64 |
| Honeybee | 33 | 12.8 | 18 | 11.2 | |

*Note*: *$p$-values calculated with chi-squared test.

**Key Messages**

- Depot preparation during VIT induction reduced risk of LLRs by 85% compared to aqueous preparation.
- Preparation type (depot or aqueous) did not influence risk of systemic reaction.
- Risk of adverse reaction linearly increased with age by 27% every decade.

In our study population, about 9% of patients experienced adverse reactions (both LLRs and SRs) during VIT build-up phase; they were more frequently experienced by patients treated with aqueous preparation (16.3%) than depot preparation (4.7%). The risk of systemic reactions was not influenced by the type of preparations. Adjusting the relative risk of adverse reactions during VIT build-up phase to potential confounding factors (gender, age, venom type, Mueller grade pre-VIT), depot preparation decreases the risk of adverse reactions of about 73%, inducing less LLRs than aqueous ones, while there is no difference in SRs incidence. These results were confirmed by multivariable analyses, where patients treated with depot preparations had an 85% decreased risk of LLR during build-up phase compared to those treated with aqueous ones. Although LLRs do not prevent reaching the protective dose of 100 μg, they are nevertheless very annoying (both for the patient and the clinician), sometimes needing the use of systemic steroids and can lengthen the build-up phase (in our centre for each LLR occurred, we readministered the previous tolerated dose). This finding can be explained assuming that depot preparations (adsorbed onto aluminium hydroxide or L-tyrosine) prevent large local allergic reactions slowing antigen presentation, without modifying efficacy. Analysing data with a multivariable Poisson model with continuous age, the risk of any adverse reaction increases by about 27% every decade, in contrast to a recent multicentre study [10].

According to literature,[9] patients treated with honeybee venom had 77% higher risk of adverse reactions, despite a wide CI. Although we excluded from statistical analysis the eight patients with mast cell activation disorders, we underline that none of them had a LLRs or SRs. To the best of our knowledge, our study has the advantage of evaluating the risk of reactions using depot preparations for VIT build-up phase compared to aqueous ones, which are more frequently used in build-up phase protocols. The main limitations of the study are: the retrospective study design, the difference in protocols used for build-up phase, the sample number, the impossibility to establish the severity of any systemic

TABLE 2 Risk of build-up phase large local reaction (LLR) according to selected variables among patients undergoing VIT, Milan, Italy

| | LLR | | Crude | | Adjusted[a] | |
|---|---|---|---|---|---|---|
| Variable | N | % | RR | 95% CI | RR | 95% CI |
| Gender | | | | | | |
| Males | 16 | 5.5 | 1.00 | Reference | 1.00 | Reference |
| Females | 9 | 7.2 | 1.32 | 0.60–2.91 | 1.39 | 0.65–2.96 |
| Age (years) | | | | | | |
| 18–39 | 0 | 0.0 | NC | | NC | |
| 40–49 | 5 | 5.3 | 0.47 | 0.17–1.31 | 0.52 | 0.19–1.42 |
| 50–59 | 11 | 11.1 | 1.00 | Reference | 1.00 | Reference |
| 60–69 | 6 | 7.0 | 0.63 | 0.24–1.63 | 0.67 | 0.26–1.73 |
| 70+ | 3 | 7.0 | 0.63 | 0.18–2.14 | 0.54 | 0.16–1.82 |
| Mueller grade pre-VIT | | | | | | |
| I | 3 | 5.1 | 1.00 | Reference | 1.00 | Reference |
| II | 4 | 6.3 | 1.23 | 0.29–5.27 | 0.93 | 0.26–3.35 |
| III | 8 | 7.5 | 1.47 | 0.40–5.34 | 1.12 | 0.36–3.53 |
| IV | 10 | 5.3 | 1.05 | 0.30–3.68 | 0.54 | 0.18–1.66 |
| Venom type | | | | | | |
| Wasp | 23 | 6.3 | 1.00 | Reference | 1.00 | Reference |
| Honeybee | 2 | 3.9 | 0.63 | 0.15–2.58 | 0.81 | 0.22–3.03 |
| Preparation type | | | | | | |
| Aqueous | 20 | 12.5 | 1.00 | Reference | 1.00 | Reference |
| Depot | 5 | 1.9 | 0.16 | 0.06–0.41 | 0.15 | 0.06–0.40 |

Abbreviations: CI, confidence interval; NC, not calculable; RR, risk ratio (from univariate and multivariable Poisson regression models with robust variance).

[a]Each variable adjusted for the others.

reaction during VIT build-up phase (the real Mueller grade of any ongoing reaction cannot be determined due to the interruption of symptoms progression by the quick administration of the emergency treatment).

In conclusion, notwithstanding these limitations, the adjusted relative risk of adverse reactions during build-up phase was 6–7 times lower with depot than aqueous preparations. Based on these results, our study supports the safety and tolerability of depot preparations used for the VIT build-up phase. Future studies are needed to confirm and better characterize our findings.

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## AUTHOR CONTRIBUTION

Pravettoni Valerio disegned the study and supplied patients. Mauro Marina supplied patients and administered venom immunotherapy, Rivolta Federica, Cappelletti Camilla, Chiei Gallo Alessandra, Sangalli Andrea gave tjheir contribution in administering venom immunotherapy and collecting the patients. Consonni Dario made the statistical analysis. Bilà Maria Beatrice gave her support as expert in venom immunotherapy supervising the paper.

## CONFLICT OF INTEREST

The authors declare they have no conflicts of interests.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Pravettoni Valerio[1]
Mauro Marina[2]
Rivolta Federica[1]
Consonni Dario[3]
Cappelletti Camilla[4]
Chiei Gallo Alessandra[4]
Sangalli Andrea[4]
Bilò Beatrice Maria[5,6]

[1]*UOC General Medicine Immunology and Allergology, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*
[2]*U.O.S. Allergology, Hospital S. Anna Como ASST Lariana, Como, Italy*
[3]*Epidemiology Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*

[4]*Allergy and Clinical Immunology Residency, University of Milan, Italy*
[5]*DISCLIMO (Department of Clinical and Molecular Sciences), Università Politecnica delle Marche, Ancona, Italy*
[6]*Allergy Unit, Department of Internal Medicine, University Hospital Ospedali Riuniti, Ancona, Italy*

**Correspondence**
Pravettoni Valerio, UOC General Medicine Immunology and Allergology. Foundation IRCCS Ca' Granda Ospedale Maggiore, Policlinico, Milan, Italy.
Email: valerio.pravettoni@policlinico.mi.it

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