

## Grade II-anaphylaxis after subcutaneous injection of mistletoe extract

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### Key words

Anaphylaxis *Viscum album*, anaphylaxis mistletoe, adverse drug reaction mistletoe, *Viscum album* case report

### Conflict of interest:

There are no conflicts of interest. David Rafei-Shamsabadi was supported by the clinician scientists program Excellent Clinician Scientists in Freiburg – Education for Leadership (EXCEL at the Medical Center - Faculty of Medicine, University of Freiburg, Germany), funded by the Else Kröner-Fresenius-Stiftung

### Case report

*Viscum album* (mistletoe) is a hemiparasitic plant with a phytopharmaceutical therapeutic effect in oncologic, cardiovascular, diabetic and inflammatory diseases<sup>1</sup>. It is also used in complementary medicine as adjunct to conventional anticancer therapies<sup>2</sup>.

We report a 33 year old female patient, treated with an unknown dose of mistletoe extract Iscador® (INCI: sodium chloride, water) for six months after surgery and radiochemotherapy for breast cancer. After each subcutaneous application she developed self-limiting urticaria at the injection site. Due to this, a different mistletoe preparation, abnobaVISCUM® Crataegi (Abnoba GmbH, Germany, INCI: sodium monohydrogenphosphate Na<sub>2</sub>HPO<sub>4</sub>, ascorbic acid, water), was prescribed by her oncologist at increasing doses of 0.01 mg, 0.02 mg and 0.2 mg. Unfortunately there was no improvement in injection site reactions. 30 minutes after the application of 1.0 mg abnobaVISCUM® Crataegi, she suddenly developed generalized urticaria, edema of the lips, tongue and eyelids with abdominal discomfort, corresponding to a grade II anaphylactic reaction (Figure 1), which responded to antihistamines and steroids. Thereafter she was sent to our Department for allergy work-up.

Prick-to-prick tests with abnobaVISCUM® Crataegi 2.0 mg (diluted 1/10 and undiluted) were negative (histamin wheel 7/20, no wheel in saline control). We additionally performed a Basophil Activation Test (BAT) with Flow CAST® (BÜHLMANN LABORATORIES AG, Schönenbuch, Switzerland) incubating the patient's blood with increasing doses of mistletoe extract. The BAT yielded a strong concentration-dependent basophil activation, which was totally absent in healthy controls (n=3). No difference was seen in the activation status of basophils in the two stimulation controls, i.e. anti-FcERI and fMLP, or the negative control between patient and control sera (Figure 2). Unspecific activation induced by Na<sub>2</sub>HPO<sub>4</sub> was ruled out (Figure 3).

Side effects such as elevated body temperature, local symptoms and headache are common during treatment with *Viscum album* but only few cases of severe reactions have been described so far<sup>2,3</sup>. Bauer<sup>3</sup> reported a case of anaphylaxis after mistletoe injection and a positive reaction in skin prick and *in-vitro* tests with basophils suggesting an IgE-mediated reaction. Hutt<sup>4</sup> described several cases of anaphylaxis after mistletoe injection with positive reaction in skin prick and intradermal tests in two instances. Pichler<sup>5</sup> reported an anaphylactic reaction after mistletoe without any provoked reaction in the performed skin tests but a positive reaction to mistletoe in lymphocyte proliferation test, suggesting there was an activation of the complement system.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the [Version of Record](#). Please cite this article as doi: [10.1111/cod.13880](https://doi.org/10.1111/cod.13880)

## Discussion

Our case proves that mistletoe extract can cause systemic anaphylactic reactions. Although we could not detect any sensitisation to *Viscum album* in the performed skin tests, we found a strong concentration-dependent basophil activation in the BAT, suggesting the prevalence of antigen-specific IgE leading to basophil degranulation. An unspecific activation by carrier substances  $\text{Na}_2\text{HPO}_4$  was ruled out, ascorbic acid has been found to have anti-allergic effects on basophils degranulation<sup>6</sup>. Humoral immunoreactivity of mistletoe was demonstrated by Klein et al.<sup>7</sup>, measuring IgG-antibodies against the major components of mistletoe, lectins and viscotoxin. In contrast we could demonstrate an IgE-mediated reaction. Although severe allergic reactions to *Viscum album* are rare additional testing, e.g. including the BAT, should be considered in suspected cases of anaphylaxis with negative skin tests.

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## Figure legends

### Figure 1.

Marked urticaria at the injection site.

### Figure 2.

#### Basophil activation test showing a dose dependent sensitization to mistletoe extract

a) Percentage of activated basophils in patients' blood compared to control. b) Pooled data of three controls (without basophil activation) and duplicate testing of patients' blood with dose dependent basophil activation. FcERI = high-affinity IgE receptor. fMLP = N-formyl-methionyl-leucyl-phenylalanine. neg = negative control.

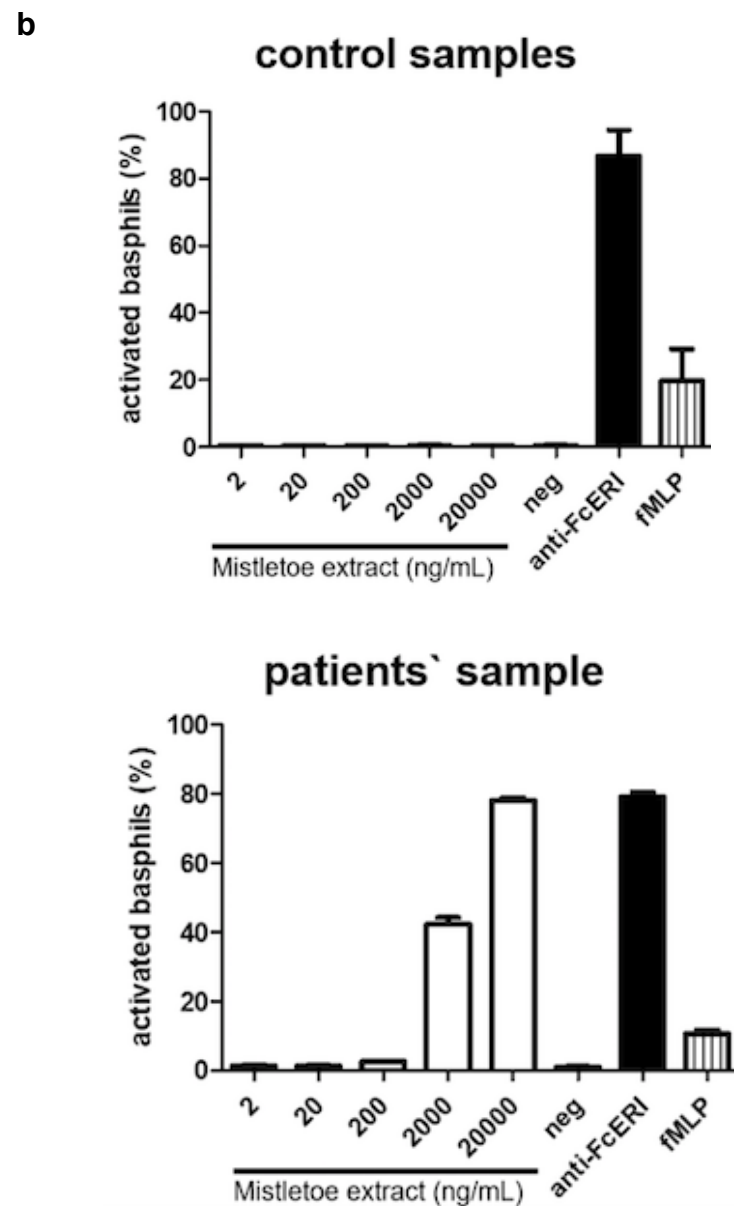
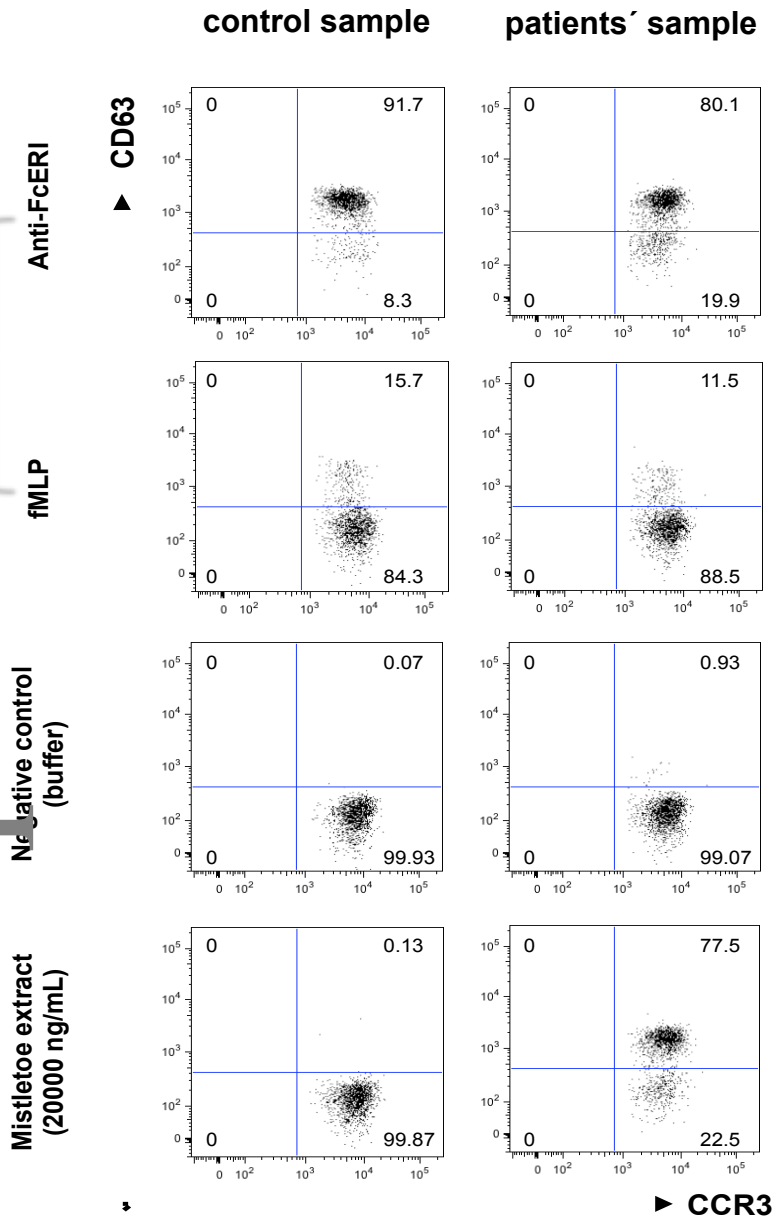
### Figure 3.

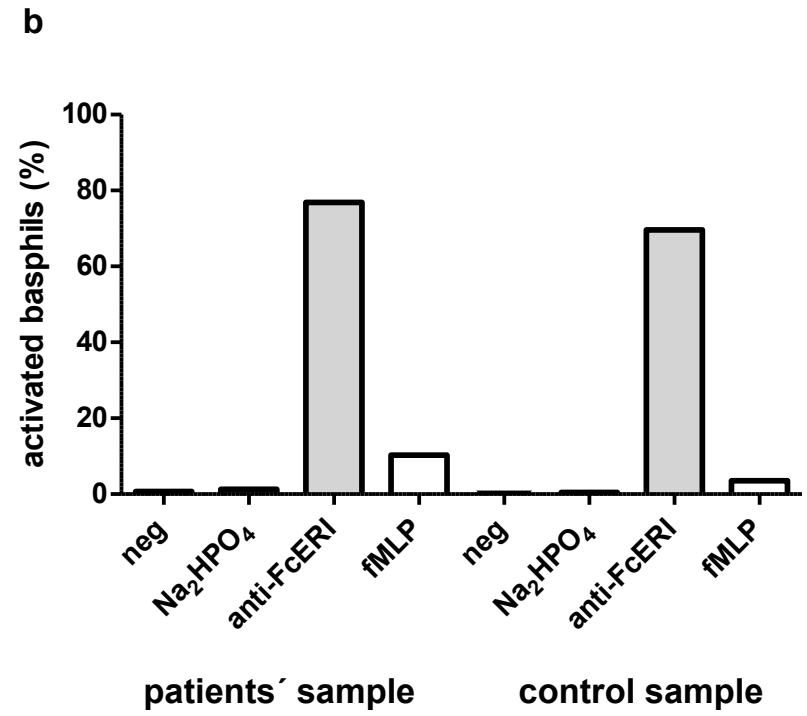
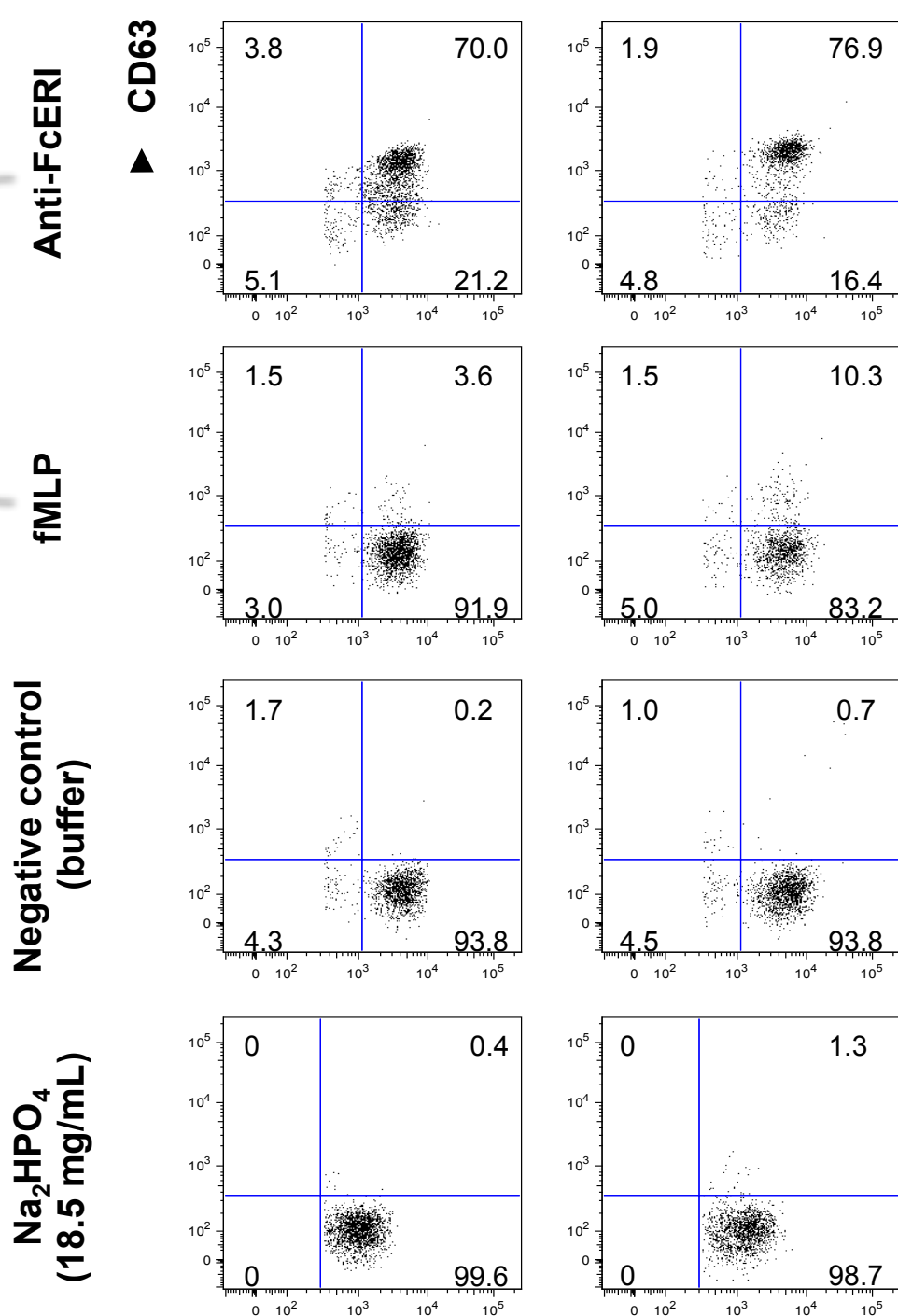
#### Basophil activation test showing no effect of $\text{Na}_2\text{HPO}_4$ on basophil activation in patient or control blood.

To rule out an unspecific activation by  $\text{Na}_2\text{HPO}_4$  which serves as a buffering substance in abnobaVISCUM Crataegi a basophil activation test (BAT) (Flow CAST®, Bühlmann Laboratories) was performed with a blood sample taken from the patient and a healthy control. a) Dot plots and b) column graphic showing no unspecific activation of basophils when  $\text{Na}_2\text{HPO}_4$  was added to the stimulation buffer in patient blood and control blood, respectively. FcERI = high-affinity IgE receptor. fMLP = N-formyl-methionyl-leucyl-phenylalanine. neg = negative control. 18.5 mg/mL of  $\text{Na}_2\text{HPO}_4$  is the concentration in 1mL abnobaVISCUM Crataegi according to the manufacturer.



60x61mm (150 x 150 DPI)





### **Basophil Activation Test (BAT)**

The Basophil Activation Test Flow CAST® (BÜHLMANN LABORATORIES AG, Baselstrasse 55, CH - 4124 Schönenbuch, Switzerland) was performed according to the manufacturer's guidelines. In brief, blood samples from the patient and three healthy controls were collected into K-EDTA venipuncture tubes. Cell stimulation was performed immediately. For each blood sample three controls were performed containing the patient background (PB), i.e. stimulation buffer alone and two stimulation controls, i.e. treatment with anti-FcεRI antibodies and N-formyl-methionyl-leucyl-phenylalanine (fMLP). Furthermore, the samples were incubated with ascending concentrations of the mistletoe extract (abnobaVISCUM® Crataegi) prediluted with stimulation buffer. The Stimulation buffer and staining reagent were added to each tube and samples were incubated for 15 minutes at 37°C in a water bath. Subsequently, pre-warmed lysing reagent was added to each tube followed by gentle mixing. Samples were then incubated for 5 -10 minutes at 18-28°C and then centrifuged for 5 minutes at 500 x g. The supernatant was decanted and the cell pellet was resuspended with 300 µl of Wash Buffer. Flow cytometry analysis was performed immediately. We gated on CCR3+ basophils and then we measured the percentage and absolute numbers of CD63+ cells within this population. The patients' blood sample was run in duplicates. Blood from three different healthy donors served as controls.

Test quality control: a) negative control (buffer control): Individual base value of 2.0-2.5 % of activated basophils is regarded as negative. b) positive control (stimulation control): two different positive controls are included (anti-FcεRI mAb and fMLP). If one of those two stimulators shows activation of >10 % basophils, the sample is evaluable.