

Flow-assisted diagnosis of anaphylaxis to hyaluronidase

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Key words: CD63; flow cytometry; hyaluronidase.

Patient 1. A 47-year-old woman was investigated for an anaphylactic reaction with pronounced angio-edema of the left face-half *ca* 30 min after infiltration of a painful scar

with a mixture of levo-bupivacaine (Chirocaïne®; Abbott, Ottignies, Belgium),

We report on two cases of hyaluronidase-induced anaphylaxis confirmed by flow cytometry.

1500 IU hyaluronidase (Hyalase®, CP Pharmaceuticals Ltd, Wrexham, UK), 50 µg clonidine (Catapressan®, Boehringer Ingelheim, Brussels, Belgium) and 40 mg triamcinolone acetonide (Kenacort®, Bristol-Meyers-Squibb, Brussels, Belgium). She was successfully treated with an antihistamine and corticosteroid, and discharged 48 h after admission. Her past medical history revealed no allergies. One month earlier she had an uneventful administration of the same compounds. Investigations for local anaesthetics, depot corticosteroids, clonidine and natural rubber latex were negative. Flow cytometric analysis (FACScan, BD, Immunocytometry Systems, San Jose, CA, USA) of activated basophils was performed using Alexa 448-coupled anti-IgE (Sigma-Aldrich, Chemic GmbH, Steinheim, Germany) and phycoerythrin-conjugated anti-CD63 (Pharmingen, BD Biosciences Erembodegem, Belgium) double labelling. The test included a negative control (without any drug), a positive control (with anti-IgE) and stimulation with hyaluronidase (serial dilution: 1/10² up to neat solution). To exclude nonspecific hyaluronidase-induced basophil activation, the test was also performed in two control individuals. Upon challenge with hyaluronidase, basophils of the patient showed a clear dose-dependent up-regulation from 1% (spontaneous CD63

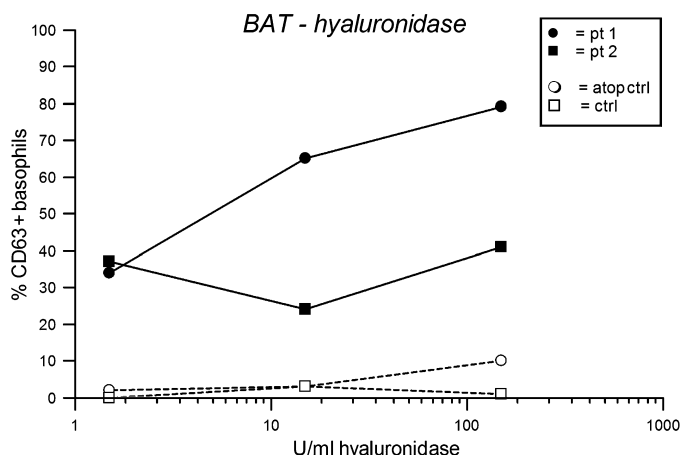


Figure 1. Percentage CD63 positive basophils in patients (pt1 and pt2) and control individuals. Ctrl and atop ctrl denote a healthy control and an atopic control suffering from inhalant allergy, respectively.

expression) up to more than 79% for the highest stimulation concentration (Fig. 1). In contrast, expression of CD63 on basophils from the control individuals remained essentially unchanged (< 10%).

Case 2. A 50-year-old woman was referred for an anaphylactic reaction with a generalized maculo-papular rash and angio-edema of the larynx within 20 min after infiltration of the lateral femoral cutaneous nerve. During that procedure, the above-mentioned mixture of chirocaïne, hyaluronidase, clonidine and triamcinolone acetonide was injected. History revealed no allergies and an uneventful infiltration with the same mixture of drugs several months before. Because the patient had several uneventful administrations of local anaesthetics and steroids, another cause, i.e. hyaluronidase was suspected. Flow cytometric analysis of activated basophils was performed as described above. As summarized in Fig. 1, upon challenge with hyaluronidase, basophils of the patient showed a consistent up-regulation from 3% (spontaneous CD63 expression) up to 41% for the highest stimulation concentration.

Hyaluronidase is a bovine testicular protein that hydrolysis hyaluronic acid, a structural component of the intercellular matrix. It is commonly used as a spreading factor in order to improve diffusion of other drugs or related compounds. Typical applications are as an adjunct to local anaesthesia, especially in

nerve blocks and ophthalmic anaesthesia (e.g. during cataract surgery), hypodermoclysis (infusion of relatively large volumes of fluid subcutaneously), as a spreading factor of chemotherapeutic agents, and as an adjunct in SC urography for improving resorption of radio-paque agents.

Hypersensitivity reactions towards hyaluronidase have been reported for more than half a century. Most described patients have demonstrated acute or delayed reactions after peribulbar anaesthesia for eye surgery (1–3). Reactions occurred at various doses and were generally characterized by gradually worsening and potentially sight-threatening orbital inflammation. However, more generalized anaphylactic reactions have also been described, and reactions might also occur from alternative routes of administration (4, 5).

In these papers diagnosis of IgE-mediated allergy to hyaluronidase was generally confirmed using skin tests (1–4) and/or quantification of hyaluronidase-specific IgE antibodies by RAST (4) or IgE immunoblot technique (5).

Upon activation, basophils up-regulate the expression of certain membrane antigens that can be detected by flow cytometry. Flow-cytometry-assisted allergy diagnosis has been proven reliable for the diagnosis of different IgE-mediated allergies including inhalant allergies, food, *hevea* latex, hymenoptera venom and drugs (for review Ref. 6).

In addition, on several occasions, the technique was demonstrated to be a confirmatory test in drug allergies where no for specific IgE are available, and also proved useful to identify cross-reactive compounds and safe therapeutic alternatives (7, 8).

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