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Biotin interference can cause false-negative specific IgE results in patients with anaphylaxis

Nils Scheib, MSc, Daniel Bauersachs, MSc, Dimitrii Pogorelov, MD, Charlotte Mara Heinrich, Feng Q. Hefeng, PhD, Carsten Bindslev-Jensen, MD PhD DMSci, Chrysanthi Skevaki, MD, Markus Ollert, MD DMSci

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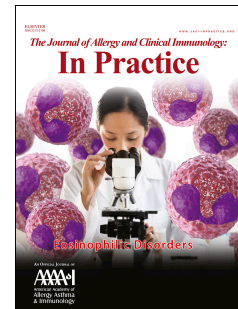
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CLINICAL COMMUNICATION

Biotin interference can cause false-negative specific IgE results in patients with anaphylaxis

Nils Scheib MSc,^{a,*} Daniel Bauersachs MSc,^{b,*} Dimitrii Pogorelov MD,^{a,*} Charlotte Mara Heinrich,^b Feng Q. Hefeng PhD,^a Carsten Bindselev-Jensen MD PhD DMSci,^c Chrysanthi Skevaki MD,^b Markus Ollert MD DMSci^{a,c,#}

^aDepartment of Infection and Immunity, Luxembourg Institute of Health, Esch-sur-Alzette, Luxembourg

^bInstitute of Laboratory Medicine and Pathobiochemistry, Molecular Diagnostics, Universities of Giessen and Marburg Lung Center (UGMLC), Philipps University Marburg, Member of the German Center for Lung Research (DZL) Marburg, Germany

^cDepartment of Dermatology and Allergy Center, Odense Research Center for Anaphylaxis, Odense University Hospital, University of Southern Denmark, Odense, Denmark

* These authors contributed equally to this work.

Corresponding author:

Prof. Dr. med. Markus Ollert, MD
Department of Infection and Immunity
Luxembourg Institute of Health (LIH)
House of BioHealth Building
29, Rue Henri Koch
L-4354 Esch-sur-Alzette
Luxembourg
Tel: +352 26970-720
Mobile: +352 621634829

Email: markus.ollert@lih.lu

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Clinical Implications

High-dose supplemental biotin can ablate allergen-specific IgE detection in sera from patients with a history of allergy/anaphylaxis on the one-step IMMULITE-2000 auto-analyzer. Thus, clinicians relying on this immunoassay should inquire about biotin intake with their patients during the visit.

Biotin is essential for human metabolism. Normal plasma biotin concentrations range from 0.1 to 0.8 ng/mL in adults consuming the recommended daily dietary dose of 30 μ g¹. The regular use of biotin-rich supplements has substantially increased among the general US adult population over the years, reaching a prevalence of 2.8% in 2015-2016². In outpatients, an even higher prevalence was noted (7.7%), while the same study revealed that 7.4% of patients in emergency departments presented with elevated plasma biotin levels above 10 ng/mL³. Particularly in patients with progressive multiple sclerosis (MS), high oral doses of biotin (100-300 mg/day) are used as adjunct treatment⁴. Both, the over-the-counter and the medically justified intake of biotin bear a considerable risk of interference in routine medical laboratory tests employing a biotin-streptavidin reaction. This can cause serious medical consequences⁵. Biotin-based immunoassays are also used for the detection of allergen-specific immunoglobulin E (sIgE) in allergy diagnosis. As previously issued in an urgent field safety notice (Siemens, IMC18-02.A.OUS) and in the subsequently updated product information, a biotin concentration exceeding 5 ng/mL in the patient samples can cause falsely depressed sIgE results on the IMMULITE platform⁵. As the potential impact of biotin interference for allergy diagnosis has not been investigated yet with clinical samples, we sought to determine the interference bias of relevant biotin concentrations in the sera of patients with sensitization to inhalant allergens or with a history of anaphylaxis in commonly-used sIgE immunoassays.

Oral doses of 20 mg biotin daily lead to a peak plasma concentration of 184 (range 80-355) ng/mL in healthy adults⁶. However, with 300 mg/day, which is used by physicians for MS, peak plasma concentrations of 824 $\pm$ 303 ng/mL were measured⁷. Therefore, we tested the potential interference by spiking sera with concentrations observed after higher dose intake of biotin as either food supplement or for medical reasons (300-1,200 ng/mL) (details under <https://doi.org/10.6019/S-BSST827>). We first measured sIgE levels in sera of 14 patients sensitized to inhalation allergens (for details, refer to **Table E1**). As controls without interference, serum from the same patient received diluent only. Patients were stratified according to lower (n=5), intermediate (n=4) or higher (n=5) sIgE levels for each allergen. We compared two biotin-streptavidin-based diagnostic platforms (3gAllergy IMMULITE-2000 and NOVEOS, utilizing a one- and two-step protocol, respectively) with ImmunoCAP that lacks a biotin-streptavidin step. In the NOVEOS and ImmunoCAP assays, no biotin interference of sIgE readouts was observed, regardless of patients' sIgE levels (**Fig. 1a, b**). However, adding biotin resulted in a strong reduction of sIgE values using IMMULITE-2000 (mean $\pm$ SD: 92 $\pm$ 6.1%) (**Fig. 1c**). Biotin at 300 ng/mL was sufficient to cause a major reduction

in sIgE signals (**Fig. 1c**), rendering all results of low sIgE samples false-negative at the 0.35 kU/L threshold (**Fig. 1d**). When the lower threshold of 0.1 kU/L was applied, at least three of five samples turned out negative (**Fig. 1d**). In patients with intermediate sIgE levels, half of the samples became negative at 300 and 600 ng/mL biotin at the 0.35 kU/L cutoff, while all samples were affected at 1,200 ng/mL biotin. At the threshold of 0.1 kU/L, only one sample was false-negative (**Fig. 1e**). Sera with higher sIgE levels were affected by biotin through a lowered sIgE result, but not with regard to a positive/negative test result (**Fig. 1f**). Biotin interference was independent of the sIgE allergen specificity (**Fig. E1**).

We then proceeded to investigate biotin interference on the two sIgE assays involving biotin-streptavidin reactions in a cohort of patients with history of anaphylaxis ($n=18$), where the accuracy of laboratory diagnosis is crucial as erroneous results may cause fatality. We included six cases each with anaphylaxis to either cashew or peanut ingestion, all confirmed by positive oral food challenge tests, and six cases of anaphylaxis after a yellow jacket sting, in whom venom immunotherapy was prescribed. Consistent with inhalant allergens (**Fig. 1**), adding biotin to sera of anaphylactic patients revealed a strong biotin interference with the sIgE readouts in IMMULITE-2000 (mean reduction 86% and 92% for 184 ng/mL⁶ and 500 ng/mL⁷ biotin, respectively) (**Fig. 2a**). Strikingly, at the 0.35 kU/L threshold, five of six tested cashew anaphylaxis patients showed false-negative results at both doses of biotin (**Fig. 2b**). We obtained false-negative results in three and four of six samples of yellow-jacket sting anaphylaxis with the addition of 184 and 500 ng/mL of biotin, respectively (**Fig. 2c**). Biotin at 184 ng/mL caused false-negative results in half of the peanut-anaphylactic patients, while a higher dose enhanced the false-negative number to five (**Fig. 2d**). To reflect that biotin interference might be dependent on the sIgE detection threshold, we calculated the cumulative percentages of false-negatives for varying sIgE cutoffs at a biotin concentration of 184 ng/mL for IMMULITE-2000 (bar diagrams, **Fig. 2b-c**). These results show that lowering the sIgE threshold to 0.1 kU/L will reduce, but not eliminate the potential biotin bias, especially in cashew- and yellow jacket-allergic patients. In an integrated analysis of all 18 anaphylactic patient samples, we observed a significant interference of both biotin concentrations on sIgE values (**Fig. 2e**) ($P\text{-value} = 8.8\text{E-}17$ and $2\text{E-}16$ for 184 ng/mL and 500 ng/mL biotin, respectively, t test using Satterthwaite's method, lmer from R). In the NOVEOS assay, the tested biotin concentrations had no effect in the 18 anaphylactic patients (**Fig. 2a, f-h**).

We demonstrated in sera of two different cohorts of allergic patients that excess of biotin can lead to false-negative sIgE results in the one-step biotin-streptavidin-based IMMULITE-2000

assay. To avoid a potential negative impact for patients, laboratories using IMMULITE and connected physicians need to be aware of detailed medication or dietary history related to biotin intake prior to sIgE testing. In this case, a discontinuation of biotin for 3-4 days should be recommended based on its elimination half life⁸. If discontinuation is not possible for medical reasons, the *a-priori* informed laboratory has several mitigation strategies to circumvent biotin interference⁹. Although our *in-vitro* spiking experiments demonstrated a biotin bias in sIgE testing using clinical samples, future studies should also analyze sera of allergic patients at different time intervals following oral administration of biotin supplements.

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Conflict of Interest

M.O. has been a consultant for HYCOR Biomedical, Siemens Healthineers and Hitachi Chemical Diagnostics; and a speaker for Thermo Fisher Scientific, Siemens Healthineers, HYCOR Biomedical and Allergy Therapeutics. C.S. is a consultant and receives research funding from HYCOR Biomedical, Bencard Allergie and Thermo Fisher Scientific; receives research funding from Mead Johnson Nutrition (MJN). The other authors declare no conflict of interest.

Figures

Figure 1. Biotin concentration-dependent interference in sIgE immunoassays testing sera from patients sensitized to inhalant allergens.

Sera were from patients with lower (d), intermediate (e) or higher (f) sIgE levels. Biotin-spiked sera were analyzed in parallel on three indicated immunoassays (a-f). Green or blue digits represent the number of samples below the threshold of 0.35 kU/L or 0.1 kU/L (d-f).

Figure 2. Biotin interference in sIgE immunoassays testing sera of anaphylactic patients.

Green digits represent the number of false-negative sIgE values (b-e; f-h). Cumulative distribution percentages for varying sIgE cut-off values measured by IMMULITE-2000 within each indicated allergen group (b-d). Diagnostic threshold, 0.35 kU/L. P-values were determined by a linear mixed model (***P<=0.001, lmer from R).

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