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**IgE-mediated anaphylactic reaction against free synthetic folic acid and methyl folate**

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### ***Clinical Implications box***

We report two cases with immediate type reactions to synthetic folic acid and methylfolate tablets and folic acid-fortified cereals and identification of specific IgE against free folic acid. Folic acid sensitization should be carefully considered in patients taking supplements and enriched food products and in those with suspected cereal allergy.

### **To the Editor,**

Folate is the generic term for vitamin B9, which is notably found in leafy vegetables, dried beans and peas and bakers yeast. Folic acid (FA) is a fully oxidized, synthetic form of folate not naturally present in foods (Figure 1). FA is used as supplement for the treatment of folate deficiency and the prevention of birth defects and chronic diseases.<sup>1</sup> The mandatory food fortification with FA has been introduced in the USA and Canada for the last 3 decades.<sup>1</sup> It has been extended to other countries but not in Europe. FA is also added in many multivitamin drinks and cereal derived food products. Hypersensitivity to FA present in tablets and drinks has been described in very few cases through mechanisms that are not clearly established.<sup>2-5</sup>

We report two cases with immediate allergy to FA and we found specific IgE against free FA in one of the two cases. The first case was a 34-year-old Italian woman who had an anaphylactic reaction 5 minutes after taking Fertifol (a 400 µg FA tablet), with angioedema, vomiting, dyspnea, lipothymia and spontaneous resolution after 40 minutes. One month later, she had a more severe reaction with angioedema, dyspnea, hypotension, tachycardia and lipothymia less than 5 minutes after taking PreFol (a 400 µg methylfolate tablet). The episode was resolved after treatment with antihistamine, betamethasone and adrenaline. Intradermal testing with FA was positive (5 µg/mL, 10 mm wheal, 45 mm flare). The second case was also an Italian female, aged 30 years. She had an anaphylactic reaction with urticaria, dyspnea, tachycardia, hypotension and lipothymia 60 minutes after taking a tablet of 5 mg FA (FOLINA® Acido folico, Teofarma, Italy). She was admitted to emergency room where she received adrenaline therapy. One month later, she had an extensive erythematous rash 120 minutes after ingestion of Kellogg's brand cereals fortified with FA. We observed a positive skin-prick test with FA (5 µg/mL, 14 mm wheal, 35 mm flare) and positive intradermal skin testings with methyl folate (100 µg/mL, 12 mm wheal, 40 mm flare), folinic acid 10 µg/mL (9 mm wheal, 29 mm flare) and methotrexate (150 µg/mL, 12 mm wheal, 35 mm flare). We

subsequently assessed the presence of specific serum IgE against FA by fluoro-enzyme Immunoassay (FEIA) in this second case. The serum of the first case was not available. We prepared FA covalently coupled to epoxy-activated Sepharose 6B, as previously described.<sup>5</sup> We detected specific IgE bound to folate-Sepharose using a secondary enzyme-coupled antibody. The results were expressed as a percentage of the anti-IgE antibody bound to the solid-phase drug. We performed a competitive inhibition assay of IgE binding to folate-Sepharose in the presence of increasing concentrations of soluble folic acid, folinic acid or methotrexate. The detection of serum-specific IgE bound on folate-Sepharose was positive (Figure 2). The binding of specific IgE to folate-Sepharose was significantly inhibited by folic acid and to a much lesser extent by methotrexate and methylfolate (Figure 2).

The mode of sensitization of patients with folate allergy is still a matter of debate.<sup>2-5</sup> Our two patients had no reported previous exposure to anti folate drugs, folate tablets or multivitamin drinks. The synthetic folic acid is more prone to act as an allergen than natural folate isoforms. The FEIA inhibition test suggested that the patient's specific IgEs had a much higher affinity for FA than for reduced folates (Figure 2). Natural polyglutamated reduced folates are transported as monoglutamate folate in blood and stored as polyglutamylated folate in tissues and erythrocytes (figure 1). FA can be absorbed more rapidly than natural folate.<sup>6</sup> Unmetabolized FA is detected in blood, in subjects who use FA supplements and folate-rich dietary products.<sup>7</sup> An association between blood unmetabolized FA and NK cytotoxicity suggested an effect of high FA intake on immune function in postmenopausal US women.<sup>8</sup> In addition, FA can bind to folate receptor beta of pro-Th2 M2 anti-inflammatory/regulatory macrophages (Figure 1).<sup>9</sup>

All previously reported allergic reactions against FA were produced by tablets and fortified drinks<sup>2-5</sup>. The report of our second case shows that they can also occur with FA fortified cereals. FA can be recognized as an allergen by mucosa as it triggers reactions few minutes after ingestion. Despite limited *in vitro* cross reactivity of IgE with reduced folates (Figure 2), the FA sensitization produces an *in vivo* cross reactivity illustrated by the consecutive anaphylactic reactions against FA and methyl folate in the first case and the cross reactivity between FA, methyl folate and folinic acid in skin tests of the second case. The cross reactivity of FA with methotrexate suggested that patients with FA allergy should be considered at increased risk of sensitivity to methotrexate and related drugs and conversely, that patients with immediate-type reactions to methotrexate and pemetrexed could be at increased risk of reactions against FA from supplements and fortified food.

A previous group identified serum IgE that recognized FA covalently coupled to human

serum albumin (HSA) but not free FA.<sup>3</sup> They suggested that FA could act as an allergen through its haptenization with self-proteins.<sup>3</sup> Our FEIA data provide the first evidence that FA allergy can be related to an IgE-mediated mechanism against free FA. These results are consistent with the absence of reported haptenization of folate in human metabolism and the release of basophile histamine by free FA in another case of FA allergy.<sup>1,4</sup> In fact, drugs with similar molecular size than FA, including muscle relaxant drugs, can produce IgE-mediated sensitization without any haptenization.<sup>6</sup>

To our knowledge, we report the first patient who underwent an allergic reaction after ingestion of food derived from FA-fortified cereals. This reaction was weaker than that produced by folic acid tablets. In regard to the few cases reported in the literature, FA sensitization may be under estimated. It should be investigated not only in patients taking supplements but also in those with suspected cereal allergy and negative skin testing with FA-free cereal extracts.<sup>2</sup> The label of FA-fortified food products and the access to FA-free cereals and related food brands should be considered for FA allergic subjects.

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## Figure Legends

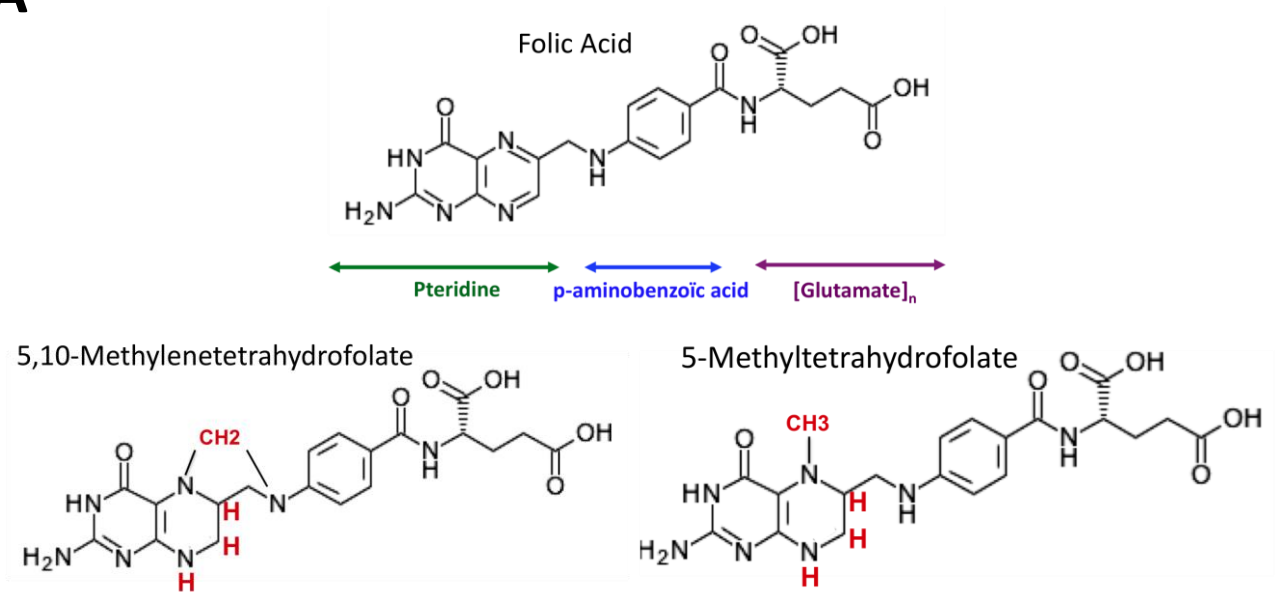
### Figure 1

**A.** Folate consists of pteridine attached to p-aminobenzoic acid and glutamate residues. The monocarbon is carried on N5 (methyl folate) and N5, N10 (folinic acid). **B.** Folate absorption and distribution. Abbreviations : folate polyglutamate (FPG) and (FMG), gamma-glutamyl carboxypeptidase (FGCP), dihydrofolate reductase (DHFR), methyltetrahydrofolate (methylTHF), folate binding proteins (FBP), folate receptor (FR), reduced folate carrier (RFC), proton-coupled folate transporter (PCFT).

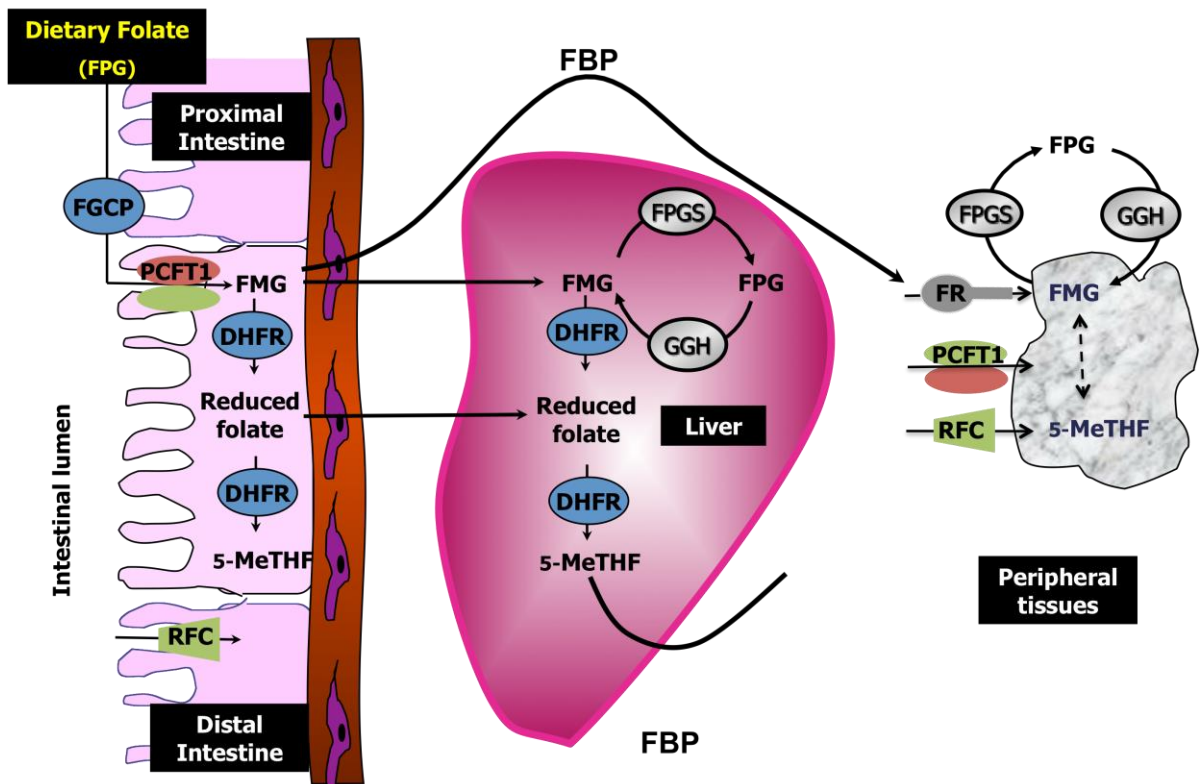
### Figure 2

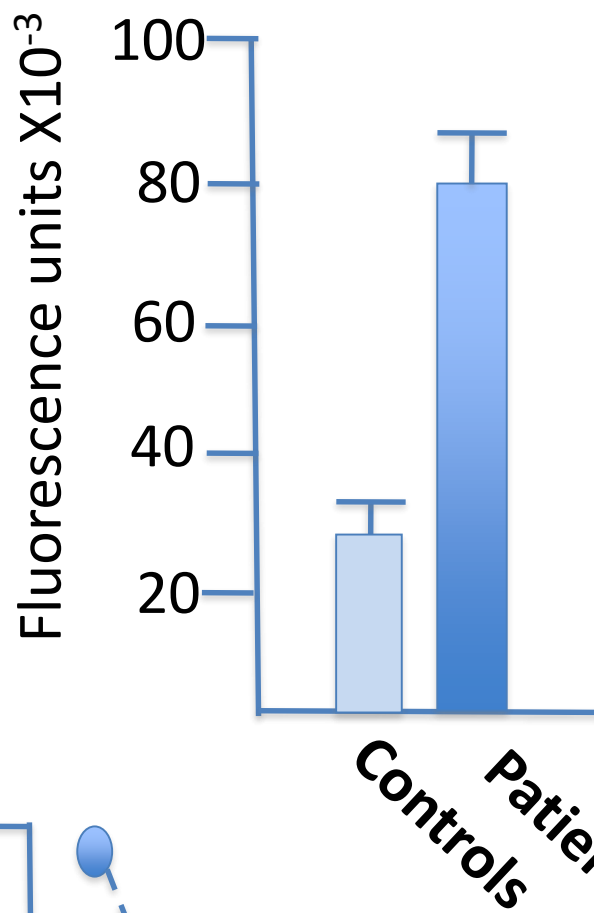
IgE detected by folic acid-Sepharose fluoro-enzyme Immunoassay in controls (n = 6) and patient (4 determinations). **A.** Specific IgE level is reflected by fluorescence intensity of anti-IgE conjugate. **B.** IgE binding is inhibited by serum incubation with folic acid. The inhibition (I) is estimated from fluorescence intensity in presence (B) or without folic acid (A), using the formula:  $I = A - B / A$

**A**



**B**



**A****B**