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

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30 *Clinical Implications box*

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

35

36 *To the Editor,*

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

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

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

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

84 All previously reported allergic reactions against FA were produced by tablets and fortified
85 drinks²⁻⁵. The report of our second case shows that they can also occur with FA fortified
86 cereals. FA can be recognized as an allergen by mucosa as it triggers reactions few minutes
87 after ingestion. Despite limited *in vitro* cross reactivity of IgE with reduced folates (Figure 2),
88 the FA sensitization produces an *in vivo* cross reactivity illustrated by the consecutive
89 anaphylactic reactions against FA and methyl folate in the first case and the cross reactivity
90 between FA, methyl folate and folinic acid in skin tests of the second case. The cross
91 reactivity of FA with methotrexate suggested that patients with FA allergy should be
92 considered at increased risk of sensitivity to methotrexate and related drugs and conversely,
93 that patients with immediate-type reactions to methotrexate and pemetrexed could be at
94 increased risk of reactions against FA from supplements and fortified food.

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

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

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

110

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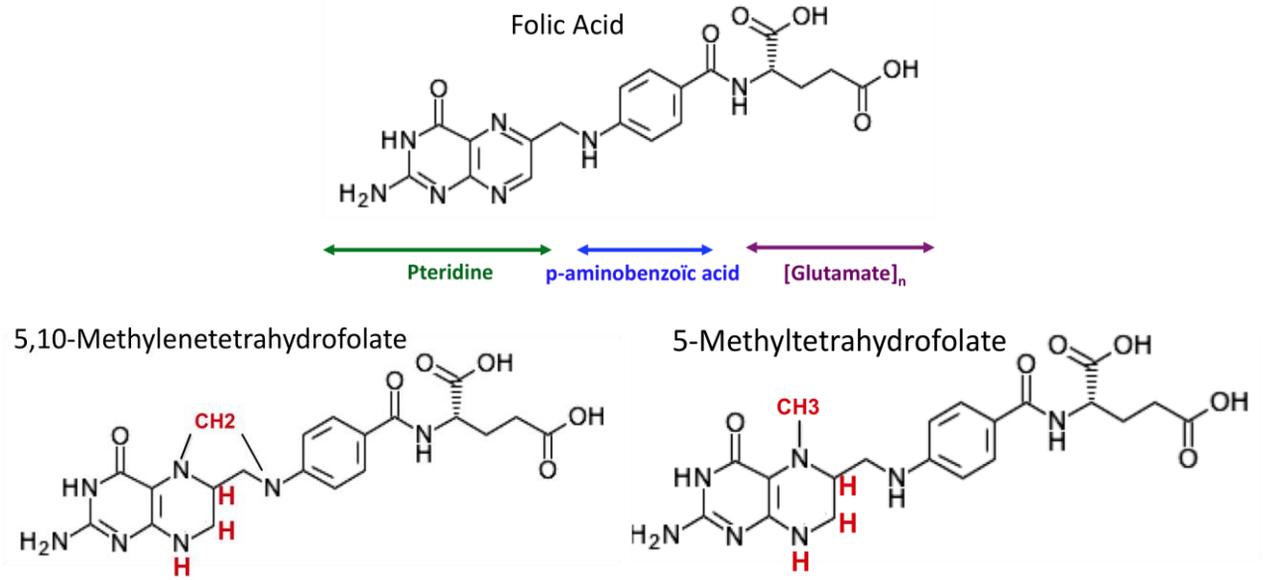
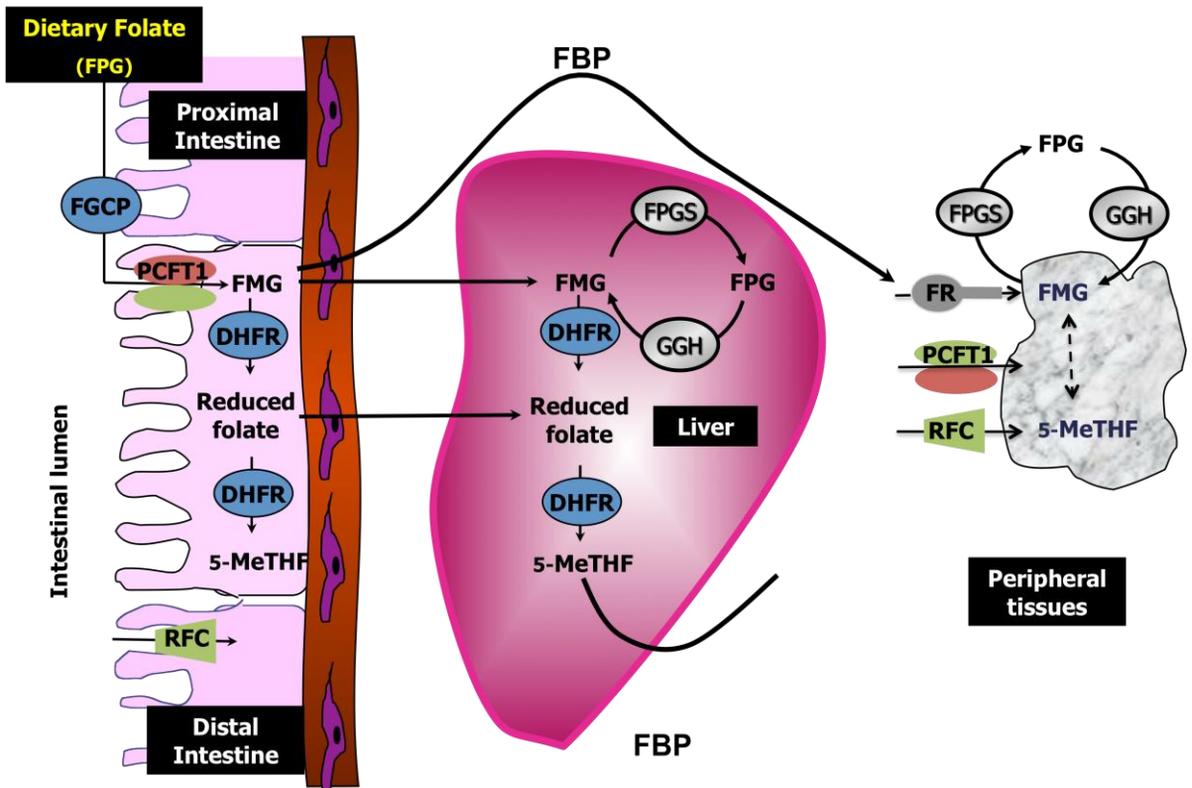
147 **Figure Legends**

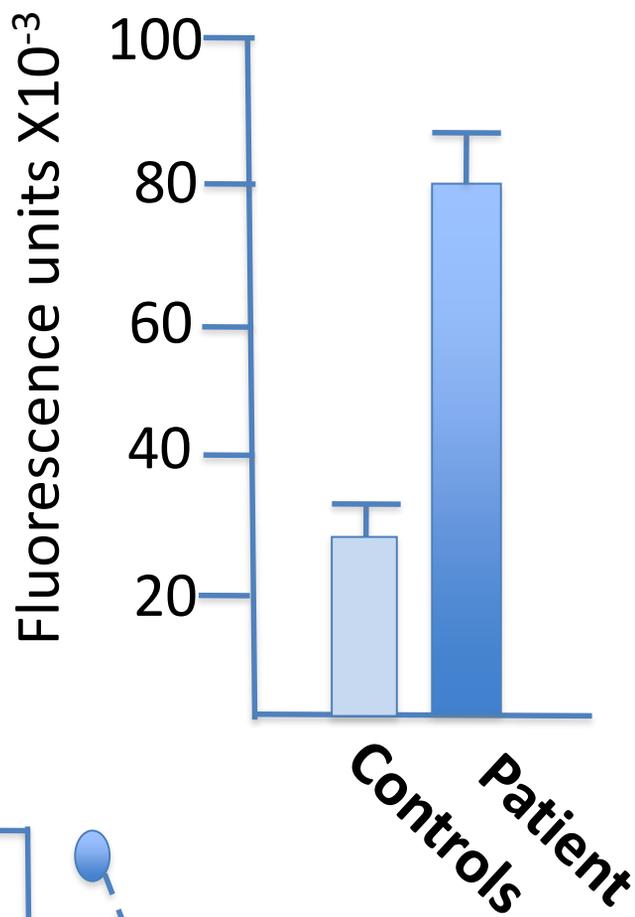
149 **Figure 1**

150 **A.** Folate consists of pteridine attached to p-aminobenzoic acid and glutamate residues. The
151 monocarbon is carried on N5 (methyl folate) and N5, N10 (folinic acid). **B.** Folate absorption
152 and distribution. Abbreviations : folate polyglutamate (FPG) and (FMG), gamma-glutamyl
153 carboxypeptidase (FGCP), dihydrofolate reductase (DHFR), methyltetrahydrofolate
154 (methylTHF), folate binding proteins (FBP), folate receptor (FR), reduced folate carrier
155 (RFC), proton-coupled folate transporter (PCFT).
156

157 **Figure 2**

158 IgE detected by folic acid-Sepharose fluoro-enzyme Immunoassay in controls (n = 6) and
159 patient (4 determinations). **A.** Specific IgE level is reflected by fluorescence intensity of anti-
160 IgE conjugate. **B.** IgE binding is inhibited by serum incubation with folic acid. The inhibition
161 (I) is estimated from fluorescence intensity in presence (B) or without folic acid (A), using the
162 formula: $I = A - B/A$
163

A**B**

A**B**