

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

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17 **Conflict of Interest disclosure statement:** the authors have no conflict of interest to declare

18 **Funding**

19 This work was supported by a grant from the french PIA project « Lorraine Université

20 d'Excellence », reference ANR-15-IDEX-04-LUE

21 **Word count:** 1000

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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.

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**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.<sup>1</sup> The mandatory food  
41 fortification with FA has been introduced in the USA and Canada for the last 3 decades.<sup>1</sup> 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.<sup>2-5</sup>

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.<sup>5</sup>  
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.<sup>2-5</sup> 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.<sup>6</sup>  
79 Unmetabolized FA is detected in blood, in subjects who use FA supplements and folate-rich  
80 dietary products.<sup>7</sup> An association between blood unmetabolized FA and NK cytotoxicity  
81 suggested an effect of high FA intake on immune function in postmenopausal US women.<sup>8</sup> In  
82 addition, FA can bind to folate receptor beta of pro-Th2 M2 anti-inflammatory/regulatory  
83 macrophages (Figure 1).<sup>9</sup>

84 All previously reported allergic reactions against FA were produced by tablets and fortified  
85 drinks<sup>2-5</sup>. 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.<sup>3</sup> They suggested that FA could act as an allergen  
97 through its haptization with self-proteins.<sup>3</sup> 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.<sup>1,4</sup> In fact, drugs with  
101 similar molecular size than FA, including muscle relaxant drugs, can produce IgE-mediated  
102 sensitization without any haptization.<sup>6</sup>

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.<sup>2</sup> 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.

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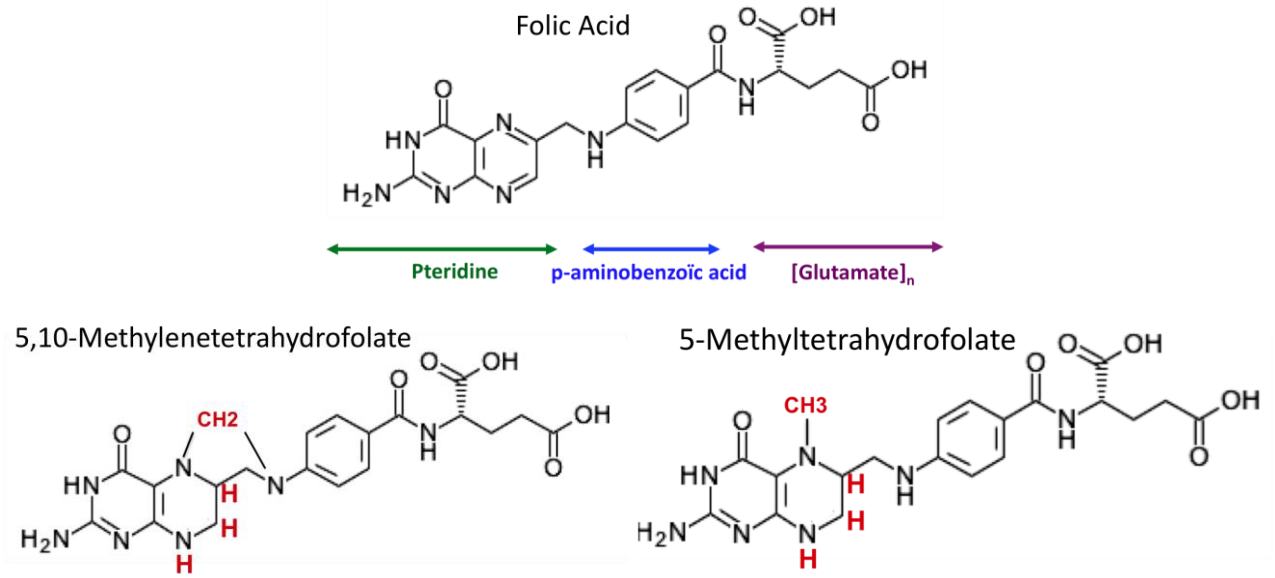
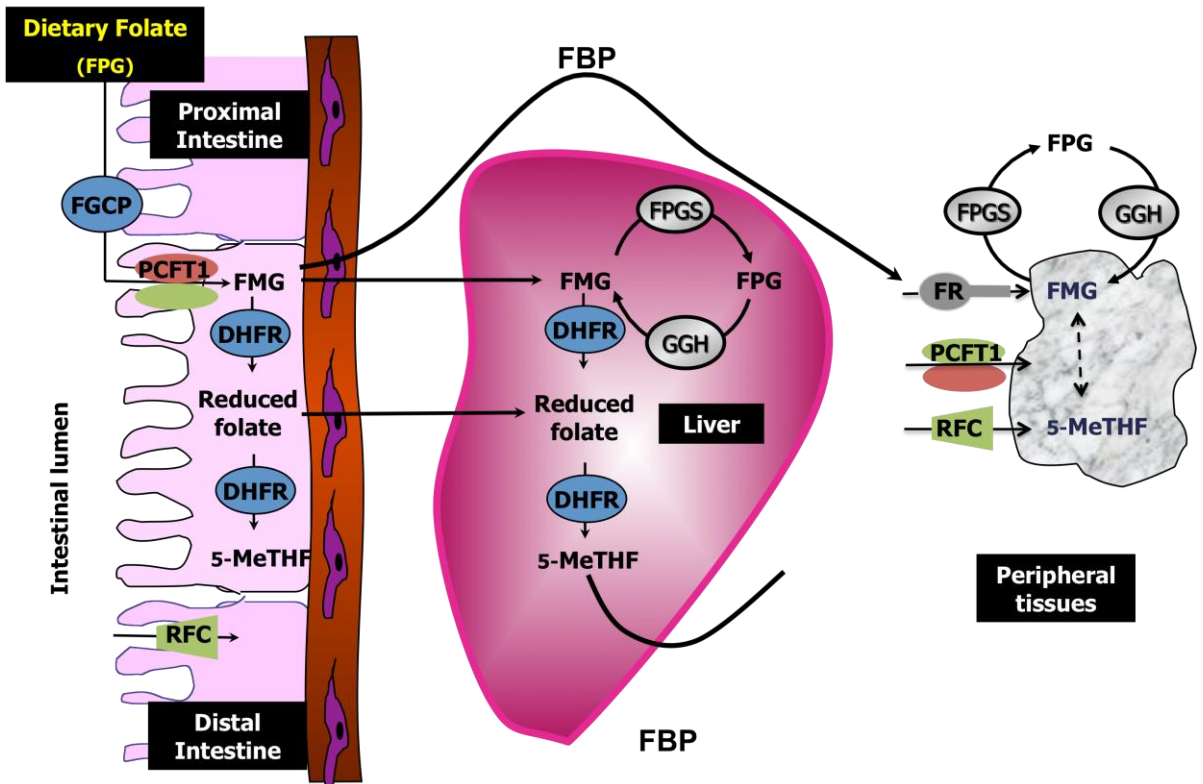
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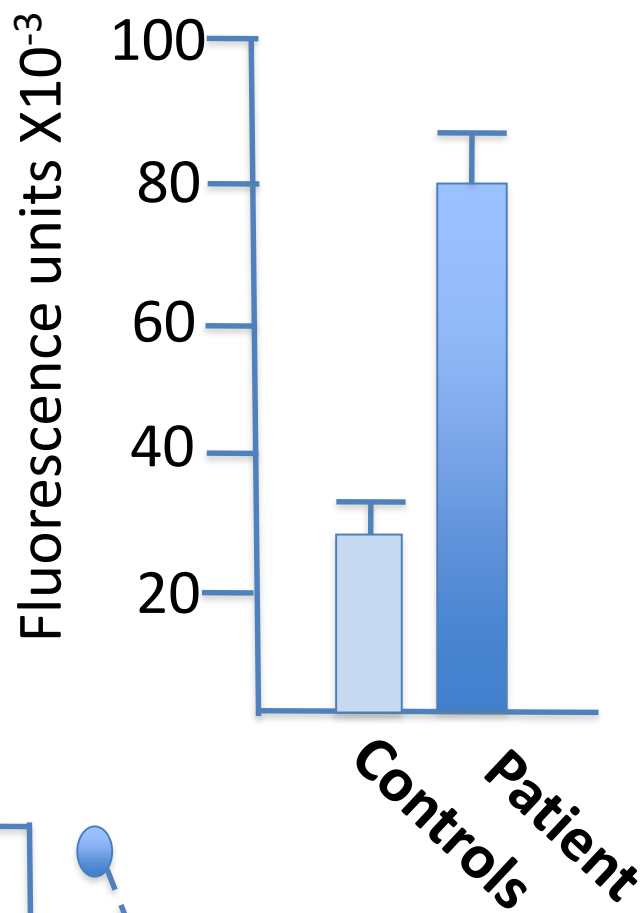
### 149 **Figure 1**

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:  $I = A - B/A$   
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**A****B**

**A****B**