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Watch the walnuts: severe immune thrombocytopenia after consumption of English walnuts

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Acute immune-mediated thrombocytopenia is a potentially serious adverse reaction and many drugs are recognised to cause this phenomenon (1, 2). A well-known example is quinine (3). Not surprisingly, patients with immune thrombocytopenia associated with drugs or food are often diagnosed as having autoimmune thrombocytpenic purpura (ITP). Thrombocytopenia associated with food, beverages, herbal remedies, or nutritional supplements is very rare, except for quinine-containing beverages. Although case reports are published of patients with histories of haematuria, petechiae or bruises after ingestion of cow's milk (4), cranberry juice (5) or pulped sesame seeds (6), a causal association is not always proven. In fact, convincing evidence for a causal association can only be given if [a] the suspected nutritional component is taken before thrombocytopenia occurred with complete recovery after discontinuation of the food, [b] all other possible causes of thrombocytopenia are reasonably excluded, and [c] re-exposure results in recurrent thrombocytopenia. To obtain definite evidence, criterium [d] must also be fulfilled, namely demonstration of anti-platelet antibodies in the patient's serum that are induced by an antigenic epitope present in the nutritional component. In this short communication, we like to present a patient case in which all criteria are met for establishing a causal association between exposure to walnuts and thrombocytopenia. Recently, this case study was published in The Lancet (7).

Patient and Methods

In January 2009, a 70 year man was referred by his general practitioner to our clinic because of an unexplained thrombocytopenia (32×10^9/L, see table 1) after a night suffering from nausea, vomiting and fever. He was not known with a haemorrhagic diathesis and did not take medications or beverages containing quinine. Platelet count recovered after 4 days. A second incidence of thrombocytopenia with systemic symptoms occurred, see table 1, and the patient recalled eating an ice cream containing nuts, where after he suggested a connection with consumption of walnuts. The patient agreed with an in-hospital challenge with walnuts to test this hypothesis. As shown in table 1, in-hospital challenge with 100 grams of English walnuts (*Juglans regia*) resulted in a deep thrombocytopenia, 15 h post-challenge. Interestingly, fever (temperature 38.7° C), nausea and vomiting again developed, 4 h post-challenge. During the next 8 h he bled from an existing small wound and large haematomas were seen at the sites of venapuncture. No other physical or systemic symptoms such as rash, angioedema or hypotension occurred. The patient was not treated with intravenous human immunoglobulin or prednisolone and time-dependent recovery of the platelets is depicted in table 1. Subsequently, the patient excluded nuts from his diet and complaints have not recurred over a 16-month period.

At the time of platelet normalisation, more patient blood was sampled to identify the presence of possible anti-platelet antibodies in the patient's serum by flow cytometry. Firstly, a saline extract (WSE) was made from ground English walnuts by emulsifying 1 gram nuts in 70 ml sterile saline. A volume of 30% BSA was added to achieve a concentration of 0.3% BSA. The WSE was centrifuged at 18,000 x g for 20 minutes, and the supernatants was used for antibody studies, according to the described procedure of drug-dependent platelet antibodies (DDAb) (8). Then, both

Table 1. Platelet counts of the patient and exposure to walnuts

Episodes	Exposure	Day	Platelet counts ($\times 10^9$/L)
A[a]	none	0	298
B	eating walnuts	1	32
B	recovery	4	212
C	eating walnuts	1	37
C	recovery	3	89
C	recovery	41	229
D	in-hospital challenge (before)[b]	0	233
D	in-hospital challenge (after)	1	4
D	recovery	2	8
D	recovery	3	27
D	recovery	4	68
D	recovery	7	205

[a] platelet count measured at routine control
[b] challenge with 100 g of walnut

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control sera (N = 4) and patient's serum (dilution 1:50) were incubated with group O platelet in the presence or absence of WSE. Samples were then washed with buffer or WSE, stained with FITC-anti-human IgG, and platelet-bound fluorescence was determined by flow cytometry. Fluorescence ratio (FLR) of median fluorescence intensity (MDFI) for serum with WSE/MDFI with buffer was calculated, and FLR ≥ 2.0 was considered positive for DDAb (8). Finally, to investigate the specificity of the anti-platelet IgG antibodies, the complete procedure was repeated using platelets of patients with type I Glanzmann's thrombasthenia, thus lacking alphaIIb/beta3 integrin (GPIIb/IIIa) (table 1).

Results and Discussion

In our hospital, provocation was done with 100 g walnuts, but in retrospect we should have used a challenge scheme starting with a very low amount of nuts and increasing the dose with small steps as the resulting complications were dangerous for the patient. With the exception of the very low platelet count, all laboratory results were normal. Furthermore, there was no eosinophilia and serum total IgE was not abnormal (129 kU/l) and no specific IgE antibodies against walnuts or other tree nuts could be measured. The rapid improvement in platelet count after each episode suggested the presence of platelet-reactive antibodies, most likely specific for a substance present in walnuts.

Flow cytometry studies were used for detection of drug-dependent antibodies reactive with platelets (8) and this study shows its power with food analogues. Figure 1 shows that strong IgG antibodies (titer 1:32) were detected in the patient's serum that reacted with platelets only in the presence of WSE. From figure 1 it can also be derived that the patient's antibody failed to react with platelets lacking alphaIIb/beta3 integrin, indicating specificity for that target protein.

As far as we can see, this is the first case in which a patient reported systemic symptoms and in which a antibody was identified reactive against platelets specific for a substance present in the implicated food. Antibodies reactive with platelets in the presence of an extract of the consumed food were demonstrated in only one possible case of deep thrombocytopenia (1×10^9 /L) after ingestion of cranberry juice, although then the particular platelet glycoprotein antigen specificity of the cranberry juice-dependent IgM and IgG antibodies could not be established (5). The findings here provide convincing evidence that this patient's thrombocytopenic episodes were caused by an IgG antibody that reacts with alphaIIb/betaIIIa integrin when a soluble substance from walnuts is present. So, for the first time all criteria (vide supra) for establishing a causal association between a foodstuff and thrombocytopenia are met: [a] acute thrombocytopenia after consumption of walnuts followed by spontaneous recovery, [b] other causes of thrombocytopenia or drugs are excluded, [c] re-exposure with walnuts gave re-induction of the thrombocytopenia, and [d] identification of walnut-dependent platelet antibodies. The only issue remains which specific epitope in walnuts is responsible for the induction of the antibodies in our patients, and studies are in progress to answer this question (figure 1).

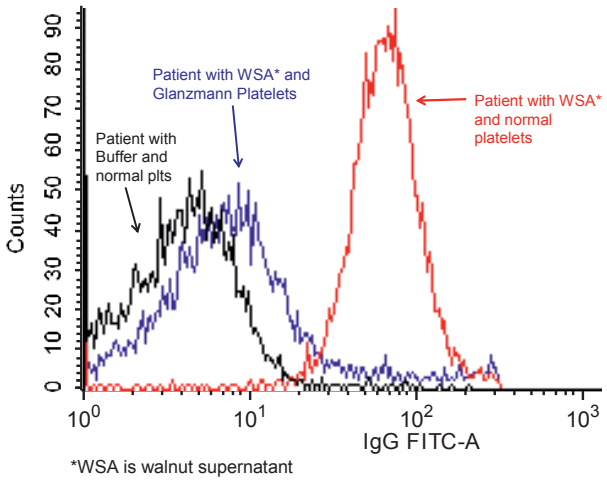

Figure 1. IgG antibodies reactive with platelets in the patient's serum as detected by flow cytometry

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