

Recognition of food-allergic patients and their allergens by the RAST technique and clinical investigation

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Summary

Sera from 119 patients with possible food allergies were tested against a panel of thirteen food allergens by the RAST technique. The results were compared with *in vivo* tests. 79% of foods causing symptoms gave a positive RAST to the specific food. Symptoms were grouped according to their time of appearance after taking the food; 'immediate' up to 1 hr and 'non-immediate' more than 1 hr afterwards. Almost all those with 'immediate' symptoms were already aware of the foods causing them and there was a 100% correlation of the RAST result with these. Only a few of those with 'non-immediate' symptoms were previously aware that these foods were responsible, and 64% of these gave a positive RAST. The majority of patients with a positive RAST result had total IgE in excess of 300 u/ml, had specific IgE antibodies against one or more common inhalant allergens, were under the age of 30 years and had a combination of asthma and eczema.

We found the RAST method a useful and safe guide upon which to base a clinical investigation of food allergy, especially for patients whose symptoms appeared more than 1 hr after the food and in whom the relationship between their symptoms and food was not apparent. The RAST technique was surprisingly successful in identifying the foods which caused these 'non-immediate' symptoms.

Introduction

In 1921 Prausnitz demonstrated that a serum factor (reagin) was responsible for Küstner's sensitivity to fish (Prausnitz & Küstner, 1921). It is perhaps a reflection of the difficulties associated with food allergy diagnosis that, until recently, this topic has been rather neglected. The physician is presented with a difficult problem because of the diverse symptoms and the lack of safe, reliable tests.

In an attempt to define food allergy, Goldstein & Heiner (1970) focused attention

on the Type I 'immediate' hypersensitivity reaction (Coombs & Gell, 1968) mediated by immunoglobulin E (IgE). However, Galant, Bullock & Frick (1973) described a group of patients who exhibited 'non-immediate' symptoms to certain foods yet, although they were seldom positive by skin testing, 48% of them had circulating food-specific IgE antibodies. The RAST—radioallergosorbent test (Wide, Bennich & Johansson, 1967)—is an *in vitro* procedure which measures circulating levels of allergen-specific IgE antibodies. It is reported to have an 80% correlation with provocation tests in inhalant allergy (Aas & Johansson, 1971). Correlations in excess of 90% were obtained when purified allergens from the cod fish (Aas & Lundkvist, 1973) and rye grass (Haddad & Hoffman, 1973) were tested. Encouraging results have been reported when the RAST technique was applied to the diagnosis of food allergies (Schur, Hyde & Wypych, 1974; Hoffman & Haddad, 1974; Chua *et al.*, 1976; Dannaeus *et al.*, 1977). However, these results have been correlated with a history of known food allergy and they have neglected those patients with unclear histories due to the 'non-immediate' onset of symptoms.

We report the usefulness of the RAST technique in recognizing food-allergic patients with both 'immediate' and 'non-immediate' symptoms, and record the clinical features helpful in identifying these patients attending a busy clinic.

Materials and methods

Patients

The 119 subjects chosen for this survey were all attending a busy outpatients clinic. The predominant complaint was asthma (sixty-six patients), but thirty-four patients had asthma and eczema, seven had mainly rhinitis and twelve had urticaria (Table 1). No attempt was made to sex- and age-match the patients; just under half gave a history of at least one known food allergy but the remainder were included because of the a possibility that they might have had a 'hidden allergy' due to food. There were sixty-five males and fifty-four females, whose ages ranged from 3 to 65 years with means of 28 and 32 years respectively.

IgE measurements

Total serum IgE was measured by a double antibody radioimmunoassay (Merrett & Pantin, 1975), with a coefficient of variation of 8.1% within assay and 10.7% between assay. Chicken was used as the source of IgE-free serum. Serum levels of IgE antibodies specific for meadow grass pollen, *Dermatophagoides pteronyssinus* and cat epithelium were measured by the Phadebas RAST® (Pharmacia Diagnostics AB, Uppsala, Sweden) to establish the atopic status of the patients (Merrett & Merrett, 1978). Specific IgE antibodies to egg, cow's milk, cod fish, cereal flours (wheat, rye, barley and oats), peanuts, nuts (hazel and brazil) and almond nuts were also measured by the Phadebas RAST®. RAST allergen discs for rice and coconut were made available for experimental purposes. Results were expressed in the Phadebas RAST® score system—0, 1, 2, 3 and 4—with 2 or more being regarded as positive.

Food elimination and challenge tests

Some patients knew that a certain food was responsible for their symptoms because these occurred very soon after taking it, but in the majority of the remainder the positive or negative food RAST result was confirmed by elimination and subsequent

Table 1. Number of patients with positive and negative food RAST results, grouped according to symptoms, atopic status, age and sex

Patients	Asthma	Asthma with episodic mouth irritation or vomiting	Asthma and eczema with episodic mouth irritation or vomiting	Asthma and eczema	Urticaria	Rhinitis	RAST positive to three common U.K. allergens	Age less than 30 years	Sex		Total
									Male	Female	
Number with positive food RAST results	10 (28%)	5 (14%)	9 (25%)	10 (28%)	1 (2.5%)	1 (2.5%)	34 (96%)	27 (75%) (15 children)	24 (66%)	12 (33%)	36 (100%)
Number with negative food RAST results	51 (61%)	0	15 (18%)	0	11 (13%)	6 (8%)	33 (40%)	36 (43%) (15 children)	41 (50%)	42 (50%)	83 (100%)

challenge with the foods. This was done by strictly eliminating a food or group of foods for a period of 3 or 4 weeks and then reintroducing it. A record was kept of symptoms, including chest symptoms and skin irritation, and in the majority serial breathing tests were also performed. Five patients underwent a complete fast for 4 days, except for glucose and water, and then gradual reintroduction of foods. Those whose symptoms might be severe were admitted to hospital for these tests.

Results

119 patients' sera were tested for circulating IgE antibodies directed against thirteen allergens and thirty-six gave one or more positive food RAST analyses.

Patient profile for positive food RAST

The food RAST-positive patients were grouped according to their main symptoms and compared with the test-negative patients (Table 1). The chance of a positive food RAST result was increased in the group with a combination of asthma and eczema as compared with the 'asthma only' group: nineteen out of thirty-four (56%) patients with the combined symptoms were RAST panel positive, compared to fifteen out of sixty-six (23%) patients who had asthma alone. There was a higher proportion of patients aged less than 30 years in the food RAST-positive group and the proportion of children under 15 years, fifteen out of thirty-six (42%), was much higher than with a negative RAST, fifteen out of eighty-three (18%).

Atopic status of food RAST-positive patients

In this study, about half the patients (sixty-seven) were positive to the atopy screen, i.e. they had circulating IgE antibodies against common U.K. allergens (grass pollen, *D. pteronyssinus* and cat dander). When screened for food-specific IgE antibodies, thirty-four of the 'atopy screen-positive' patients were food-positive, while thirty-three were negative (Table 1); thirty-four out of thirty-six food RAST-positive patients were also atopy screen-positive.

The percentage of patients with a positive food RAST was related to the total IgE levels (Fig. 1) and 88% were found to have levels greater than 300 uIgE/ml.

Sensitivity of the food RAST results

In this study 1,547 food RAST analyses were performed on sera from 119 patients. A proportion, 1044 (79%), of these food RAST analyses were also tested by elimination and challenge with the corresponding foods, as previously described, or the foods were already known to cause symptoms. These included 146 (77%) of the RAST-positive and 898 (66%) of the RAST-negative foods. The numbers of these foods causing symptoms are given in Table 2. The symptoms are grouped according to their time of onset after taking the food, with 1 hr being the arbitrary time limit separating 'immediate' from 'non-immediate' symptoms. A total of 112 foods caused symptoms, and eighty-eight of these were associated with specific IgE antibodies, an overall RAST sensitivity of 79%. Furthermore, forty-five foods caused 'immediate' symptoms and were all associated with specific IgE antibodies (i.e. a sensitivity of 100%), while sixty-seven foods causing 'non-immediate' symptoms were associated with forty-three positive RAST analyses (i.e. a sensitivity of 64%). 874/898 (97%) RAST-negative foods tested by elimination and challenge caused no symptoms.

Table 2. Numbers of foods tested by RAST and by elimination and challenge

Food RAST results														
	Milk	Egg	Fish	Hazel nut	Coco- nut	Brazil nut	Almond	Peanut	Wheat	Barley	Rye	Oats	Rice	Total
Positive														
Immediate symptoms (less than 1 h after food)	0	4	3	9	6	8	4	11	0	0	0	0	0	45
Non-immediate symptoms (1 h after food)	4	2	0	0	0	0	0	2	14	4	4	4	9	43
No symptoms	1	1	0	1	1	1	0	2	10	13	12	8	8	58
Total	5	7	3	10	7	9	4	15	24	17	16	12	17	146
Negative														
Non-immediate symptoms	12	9	0	0	0	0	0	0	3	0	0	0	0	24

Note: 874 RAST-negative foods did not cause symptoms.

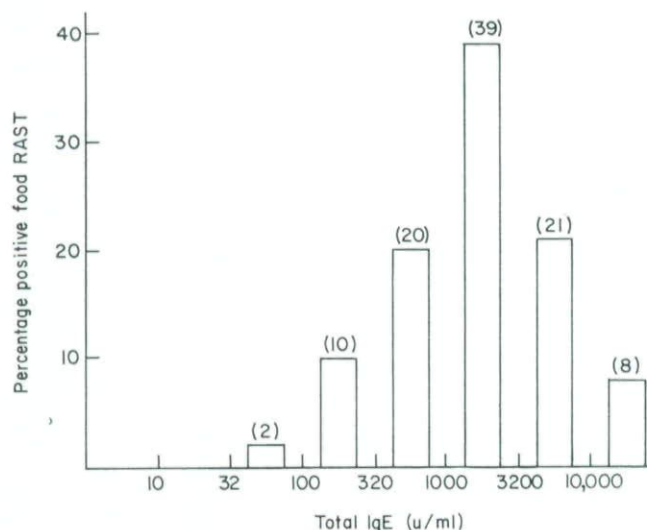


Fig. 1. Percentages of patients with positive food RAST results related to total IgE ($n = 36$).

Symptoms from foods

After excluding seven patients who had no symptoms from any of the foods tested, and two who were lost sight of, there were twenty-seven with symptoms from one or more of the positive RAST food allergens (Table 3). Twenty patients had 'immediate' symptoms from forty-five foods and eighteen had 'non-immediate' symptoms from forty-three foods, nine of the patients falling into both these categories. 'Immediate' symptoms consisted mainly of swelling or irritation of the mouth or tongue, or vomiting, and all except one of these twenty patients were already aware that these symptoms were caused by foods, principally nuts and peanuts (Table 2).

'Non-immediate' symptoms consisted mainly of asthma, but in a high proportion (Table 3) foods also caused aggravation of eczema, with skin irritation. In this group only three out of eighteen patients (nine foods) were aware of the relationship between

Table 3. Symptoms caused by foods

Food RAST results	Asthma	Aggravation of eczema with skin irritation	Asthma and aggravation of eczema	Mouth irritation, or swelling, or vomiting	Total foods
Positive ($n = 27$)					
Immediate symptoms (20 patients)	3 (6%)	7 (16%)	0 (0%)	35 (78%)	45 (100%)
Non-immediate symptoms (18 patients)	17 (40%)	6 (13%)	20 (47%)	0 (0%)	43 (100%)
Negative ($n = 18$)					
Non-immediate symptoms	9 (38%)	11 (46%)	4 (16%)	0 (0%)	24 (100%)

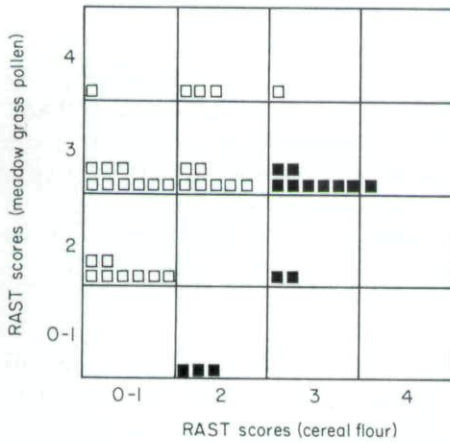


Fig. 2. Comparison of grass pollen and cereal flour RAST results in patients with symptoms from cereal flour (■) and in patients without symptoms from cereal flour (□).

their symptoms and the foods: the remaining fifteen patients were discovered to have symptoms from thirty-four of the foods, principally cereal flours (Table 2), as a result of elimination and challenge tests. These symptoms appeared from 1 to 4 hr after taking the food, the majority in about 2 hr. Twenty-two of these thirty-four foods discovered were in patients who already knew they had some other food sensitivity. However, eighteen patients had a positive food RAST result, but were without any evident symptoms from these foods. These included the seven patients mentioned above with no symptoms from any of the foods tested; five of these suffered from rhinitis and asthma due to grass pollen allergy. Table 2 shows that fifty-eight foods, principally cereal flours, gave a positive RAST result not associated with symptoms.

Fig. 2 compares the grass pollen and cereal flour RAST scores in fourteen patients with, and twenty-nine without, symptoms from the cereal. The RAST scores for pollen

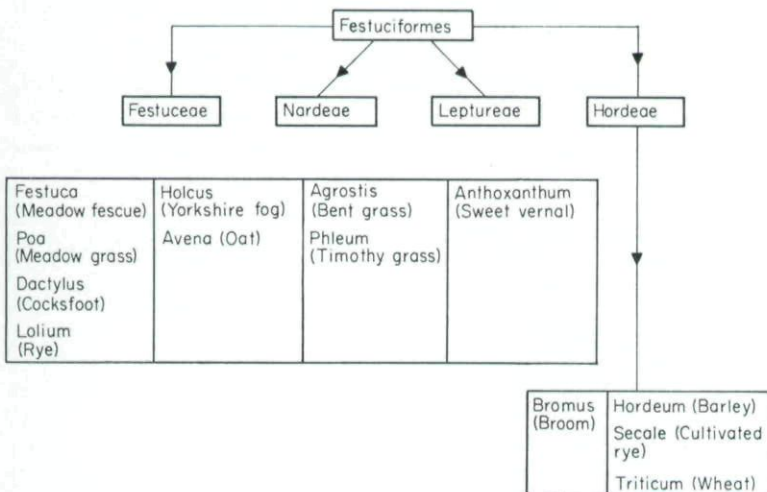


Fig. 3. A section of the *Graminae* family.

and cereal were similar, or higher for cereal, in the group with symptoms, but those without symptoms were distinguished by higher pollen than cereal flour scores. The taxonomic similarity of wild grasses and cereal grasses is shown in Fig. 3.

There were eighteen patients with 'non-immediate' symptoms from the twenty-four RAST-negative foods (Tables 2 and 3). Five of these patients had a positive RAST and symptoms from other foods. Eleven gave a positive RAST to one or more inhalants, nine of these also having a total IgE more than 300 u/ml. Eleven were aged over 30 years. Milk and egg were the principal foods. The foods caused aggravation of eczema with skin irritation, with or without asthma, more frequently than asthma alone (Table 3) and these symptoms appeared from 1 to 6 hr after taking the food.

Other symptoms

As well as the main diagnostic groups in Table 1, certain other ill-defined symptoms were apparent and improved after relevant foods were removed from the diet. Eight children initially exhibited poor appetites, low weight, irritability and poor concentration at school—all these symptoms improved when the diet was amended. Similar improvements were obtained when the diets of adults were modified; four patients initially described excessive fatigue, two had joint pains and two had diarrhoea.

Other foods and food additives

There were twenty-five patients with symptoms caused by sixty-three foods or drinks not tested by the RAST technique. These foods specifically caused asthma (thirty-seven), aggravation of eczema (sixteen), both of these symptoms (two), urticaria (four) and swelling of the mouth (four). Most already knew the foods they could not tolerate and those most frequently occurring were the pork-meat group, cheese, alcohol drinks and vinegar; also included were chocolate, chicken, soya, onion and tomato.

Eighteen patients were sensitive to colouring dyes or preservatives in foods, drinks or drugs. These were confirmed by withholding foods and drinks known to contain these substances and then challenging with them. These caused asthma (seven), aggravation of eczema (six) and both in five patients. Ten of these patients were positive to one or more inhalants of the RAST atopy screen.

Multiple food sensitivities

The group of twenty-seven patients with a positive food RAST included in Table 3 were sensitive to a total of 134 foods, including those with symptoms from RAST-positive and RAST-negative foods, and foods not included in the RAST panel (Table 4).

Table 4. Multiple food sensitivities in twenty-seven food RAST-positive patients

Number of foods	Number of patients
1	4
2	6
3-5	8
6-10	5
Over 10	4

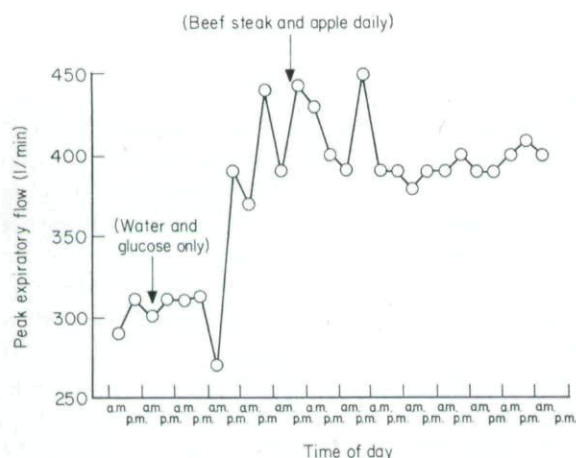


Fig. 4. Improvement in peak expiratory flow recordings after patient (A.D.) had fasted for 4 days.

Individual patient—A.D.

Male, aged 30 years. Severe asthma and eczema since infancy. Frequent admissions to hospital and requiring continuous steroids. Known sensitivities to house-dust mite and grass pollens, and to certain foods including fish, egg and nuts, which cause immediate mouth irritation and vomiting. Fasting with water and sugar only was carried out for 4 days with considerable improvement of breathing (Fig. 4). Foods were then added and it was found that wheat, rice, barley and rye each caused a significant fall in breathing test results, and each of these foods had given a positive RAST result. Fig. 5 shows the response to rice. There was considerable improvement in asthma and eczema as a result of avoidance of these foods, the use of steroids being stopped.

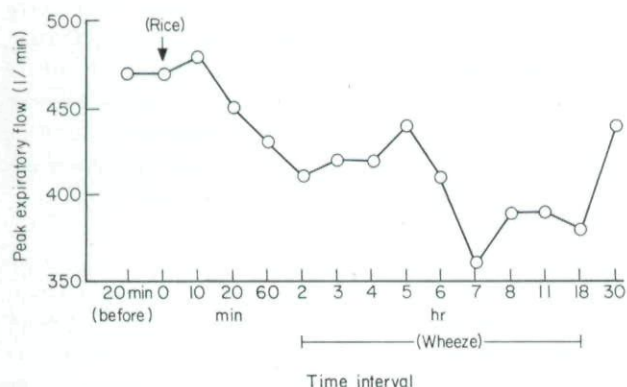


Fig. 5. Decrease in peak expiratory flow recordings after challenging patient (A.D.) with lightly boiled rice.

Discussion

Recognition of the food-allergic patients

Table 1 indicates that the people most likely to have circulating food-specific IgE antibodies will be under 30 years of age and will have a combination of asthmatic and eczematous symptoms. Similarly, from the total IgE and atopy screen data, it is most likely that a positive food RAST will be obtained when a patient in the U.K. has a total IgE level over 300 u/ml and has a positive atopy screen by RAST. The patients we found to fulfil these four criteria were likely to have one or more food allergies (i.e. IgE-mediated food hypersensitivities). Indeed, when one food allergy is known, then others should be suspected because twenty-two of the thirty-four additional food allergies discovered as a direct result of RAST food analyses were in patients already known to be hypersensitive to at least one food.

Our patients' symptoms occurred either immediately after taking a food or 1 hr or more later, and some suffered from both 'immediate' and 'non-immediate' symptoms from different foods. Generally, patients knew the foods causing the 'immediate' symptoms but, in contrast, few were aware of the foods responsible for their 'non-immediate' symptoms and it was in this group that the RAST results were found, perhaps surprisingly, to be a useful guide on which to base a clinical investigation.

Assessment of the food RAST results

The importance of a reliable *in vitro* food allergy test can be judged by considering the impracticability of performing large numbers of food elimination and challenge tests; in this study 1547 food RAST analyses were performed on sera from 119 patients and 1044 foods were proved or disproved by elimination and challenge tests, or were already known to cause symptoms. Elimination and challenge tests are necessary to confirm the food allergy but are time-consuming and require much co-operation from the patient, especially if several foods are involved. In some patients care is needed to avoid severe symptoms when foods are re-introduced so the tests cannot always be applied to outpatients. The results of the food RAST were a useful guide to elimination and challenge tests, and it was found to be quite reliable because the sensitivity was 79% and the specificity 97%.

The results confirm the promise of the RAST technique reported by others for patients with 'immediate' symptoms and extends it to that neglected group of patients with 'non-immediate' symptoms. This confirms a similar observation made by Galant *et al.* (1973), and their report that skin tests are seldom positive in patients with a 'later' onset of symptoms suggested that the RAST technique is the preferred diagnostic test in such cases. However, some explanation is necessary in those cases when (a) the existence of a circulating food-specific IgE antibody did not correlate with symptoms and (b) there was no specific IgE detected in spite of symptoms.

Food RAST analyses in asymptomatic patients

Instances of positive food RAST results in symptom-free patients were restricted to cow's milk and egg in two cases but were mainly the cases with cereal allergies. A high incidence of milk-specific IgE antibodies in milk-tolerant children had been reported previously (Foucard, 1973; Dannaeus *et al.*, 1977). Similarly, cases of egg-positive RAST results in egg-tolerant patients have also been noted (Schur *et al.*, 1974; Dannaeus *et al.*, 1977). It is possible that blocking antibodies have an inhibitory effect

in these egg- and milk-tolerant patients, but although Dannaeus *et al.* (1977) did find that children with an increasing tolerance to egg had high egg-specific IgG titres, they did not find that milk-specific IgG titres paralleled symptoms in milk-tolerant children. Therefore, the efficacy of IgG in blocking the action of specific IgE antibodies in milk-tolerant subjects seems less likely than in egg-tolerant subjects.

Gerrard *et al.* (1973) suggested that the priming of IgE-secreting cells by cow's milk protein in infancy may act as a general marker of a predisposition to atopy as well as being a specific indicator of milk allergy.

The positive cereal RAST analyses in cereal-tolerant patients is explicable either in terms of inhalant allergy or cross-reactivity, since cereals are cultivated grasses of the Gramineae family, and wheat, barley, rye and oat are members of Festuciformes, like most common British grasses. In this study, we confirm Hoffman's (1975) observation that sera may be positive to both grass pollen and cereal flours, or positive to one only, and his conclusion that the demonstration of specific IgE antibodies reacting with a cereal does not necessarily indicate a cereal flour allergy. However, it seems probable to us that a cereal flour RAST score of 2 or more will be significant when the grass pollen score is similar or less (Fig. 2).

Negative RAST analyses in patients with food intolerance

Non-immune mechanisms may explain the negative RAST results in subjects with 'non-immediate' symptoms of food intolerance, but interference in Aspergillus-specific IgE estimation by IgG antibodies has been reported (Arbesman *et al.*, 1974), and this observation might be extended to food IgE determinations. Also, in some instances, the appropriate allergen may not have been attached to the proper disc support, either because it is a non-protein or the natural food has been altered by its preparation or by digestion, or because it is absent from the natural food. For example, certain cases ascribed to egg allergy have subsequently been identified as a sensitivity to the antibiotic present in the meal fed to battery hens (Pepys & Hutchcroft, 1975). Such cases would have been egg RAST-negative—and similar detective work may have a role to play in diagnosing patients whose immediate symptoms are unconfirmed by the RAST results.

Conclusion

Adverse reactions to foods may be noticed within 1 hr ('immediate' reactions) and these are usually known to the patient. However, symptoms can occur 1 or more hr later and these mean that the causes are usually unknown to the patient. With the help of the RAST technique, and confirmation by elimination and challenge tests, a number of patients were discovered to have 'non-immediate' reactions to food—often to several foods. They were not previously aware that these 'hidden allergies' were responsible for their symptoms, which were more usually a combination of asthma and eczema. This suggests that food may play a more important part in such conditions than has been recognized hitherto.

These 'non-immediate' reactions could be a result of an IgE-mediated allergy to a food break-down product, or due to another type of immune mechanism, perhaps triggered by an IgE reaction.

In these days of 'soya steaks', 'peanut beefburgers' and food additives, it is difficult to know exactly what we do eat and drink, but once a patient is recognized as potenti-

ally allergic to foods then the results of a food RAST analysis should offer a useful guide to clinical tests and treatment.

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