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# MRT TEST - NEW GENERATION OF TESTS FOR FOOD HYPERSENSITIVITY IN CHILDREN AND ADULTS

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***Abstract:*** *In this paper results of an assessment of the diagnostic usefulness of the MRT test in 21 children between the ages of 2 to 5 years hypersensitive to cow's milk are discussed. The new feature of MRT is the possibility of detecting cell reactions to harmful antigens using an in vitro method in reference to granulocytes, lymphocytes and blood platelets. Using the test in question the method yielded the sensitivity of 94.5 percent. It was also determined that the most frequent reactions were to alfa-lactoalbumin in 85.7 percent, beta-lactoglobuline in 66.7 percent, whey proteins in 57.1 percent and casein in 47.6 percent of the patients. It was demonstrated that the differentiated cell types reacted in following fashion: lymphocytes - 38.5%; granulocytes - 47.6%; mixed reactivity (combination of lymphocytes an platelets) - 14.2%. In the control group consisting of 6 healthy children, test- negative results were found in 66.6% for the four tested antigens. In two cases MRT Test identified reactions to the fraction of alfa-globulune as high as 16.6% and beta-globuline as high as 16.5% respectively. The MRT Test seems to be useful in diagnosing levels of food hypersensitivity, possibly through detecting reactions of specific cell groups. The MTR Test demonsrated better diagnostic results then the ALCAT Test*

## INTRODUCTION

Established in 1988 as a method of evaluation of quantitative and qualitative changes in the population of granulocytes in vitro under the influence of potential harmful reactions to specific substances (allergens, toxic substances, medicines) ALCAT causes controversy in both the medical literature and simply in everyday diagnostic work. A lack of unity of identification of pathogenic mechanisms responsible for the changes( immunologic, non-immunologic) , questions concerning the reproducibility of results, the possibility of errors (false positive, false negative) and the lack of method's verification by double blind placebo controlled challenges are some of the reasons for these questions (1,11,13). According to some users the cause of problems can be traced to "…underestimating results in vitro due to detecting only the fraction of granulocytes.." (8,9,15). Both research results and clinical observations indicate that all morphological elements of blood can be involved in the end point of allergic reactions, as well as other non-immunolgic mechanisms (pseudo allergy, toxic effects, direct pharmaceutical results, etc.) (2,4,7,12 16-19). There was a need to create possible detection of changes inside the granulocytes, lymphocytes and blood platelets and their visualization (histogram). The new MRT test can accomplish the task of comparing and documenting these values..

## METHOD

Quantification of changes of total blood cell volume (granulocytes, lymphocytes, blood platelets and other solids) and volume of plasma per unit of blood volume (1mm3) is the essence of the MRT test.As a result of reactions to substances interpreted by the immune system as harmful ( e.g.. antigens), The volume of these cells changes (as a result of qualitative and quantitative changes) causing an increase in the total plasma volume (figure #1).

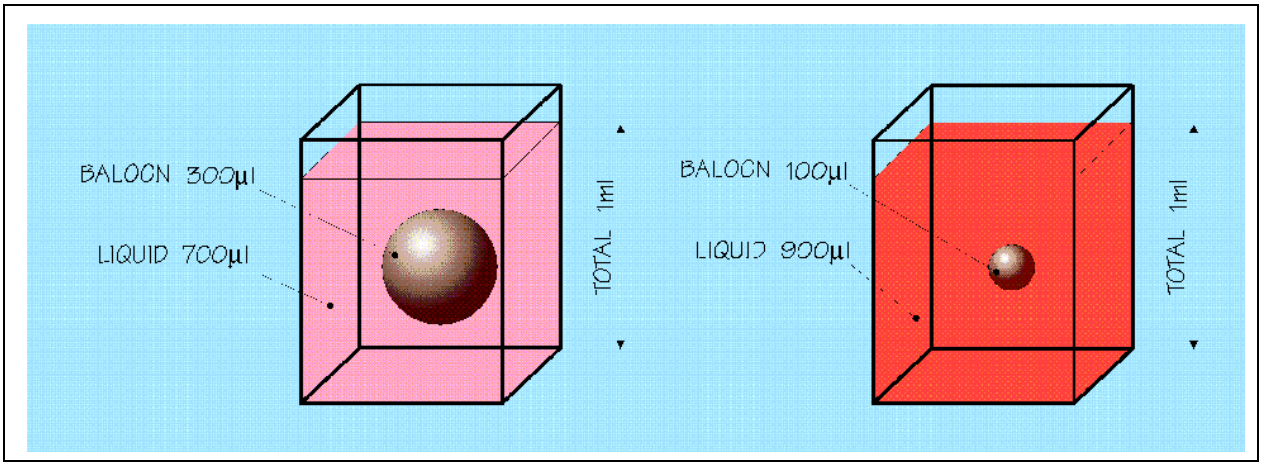


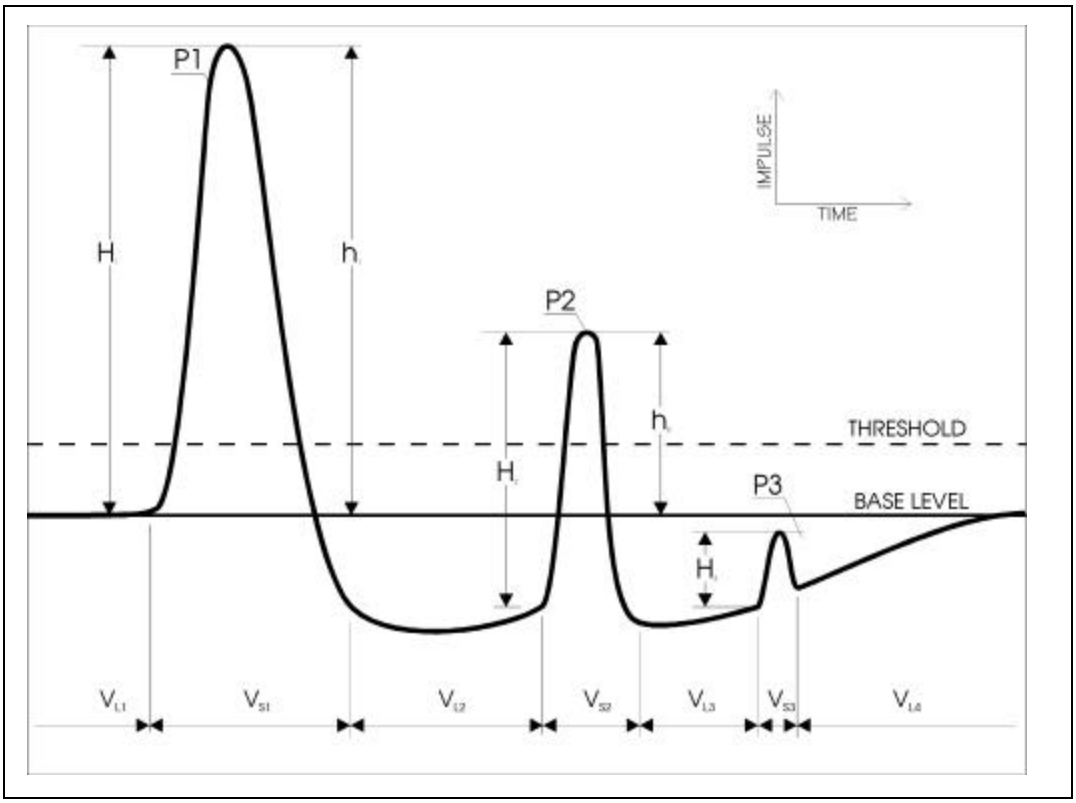

**Figure #1**

The MRT system records these quantitative changes of the cells, as opposed to Coulter application in ALCAT, which results in a count of all reactive cells (figure #2).

For the purpose of this study only to establish for comparison which groups of cells and their changes are recorded, a histographic representation of the results is used (histogram) for the MRT. The volumetric changes detected by the MRT test were converted from volumetric changes (expressed in pikoliters) to "percentages of change" similar to the ALCAT method. Registered volumetric changes of 0% - 1.3% changes were established as negative. Higher than 1.3% volumetric change was considered positive.

The master control is the same (patient) blood sample as that evaluated, without any substance exposure (allergen, medicine, toxic substance) in the medium. The first examinations of this study revealed that after incubation of the blood with the antigen, the changes of volumes of lymphocytes, granulocytes or blood platelets were observed. They can react individually or simultaneously.

**Figure #2**




**MATERIAL AND METHODS**

To evaluate the diagnostic usefulness of the MRT test, 21 children age 1-5 with diagnosed allergy to cow's milk protein were studied. Diagnosis of the allergy was established on the bases of positive skin test with the drop of cow's milk (prick test); labial test (lip test); specific IgE (s-IgE-class2 and above) antibodies for cow's milk protein were measured and verified by double blind placebo controlled oral challenge with milk. The children stayed on an elimination diet without milk from 6 to 12 months from the date of diagnosis. A control group of six (6) adults with no complaint of any clinical discomfort; no personal nor family history of atopy, and in whom the total IgE plasma level was normal was selected. They had been consuming milk on a regular basis. To evaluate sensitivity of the MRT to cow's milk reactivity, the companys' (Signet Diagnostic Corporation) reagents were used. These included custom prepared allergens of cow's milk fractions consisting of the following subgroups: casein, alpha-lactoalbumine, beta-lactoglobuline and whey proteins (extracts from Sigma Co.) The patients blood was coded and blind to the investigator and the technologists. Blood was prepared similar to the ALCAT Method, and the laboratory procedure and the tests were conducted according to the MRT protocol. Afterwards, (for an easy comparison), the results of MRT Test were converted from the volumetric (picoliters values), to the "ALCAT format". After the investigators receivied the graphic and percentage results of the whole group, decoding was done with the investigator assigning the subjects name to proper results. MRT conversion values showed that the percentage change of volume of score of 1.3% or higher corresponded to a positive result (positive detection). Similarly, the subjects test results were identified as positive if at least one antigen of cow's milk protein in the studied sample was test-positive via MRT.

(As stated previously), in order to compare the results of the MRT Test to ALCAT Test, a conversion modification of the MRT Test result algorithm was implemented. Again, the volumetric results of the MRT Test were altered to reflect "percentage change in volume". A verification study consisted of 30 adults and children known sensitive to

food. The MRT Test and ALCAT Test were conducted using a 12 antigen panel of foods ( Signet Diagnostic System Co.). The results went through the clinical-laboratory analytical comparison and were evaluated by mathematical-statistical method as shown in (Tab 5).

## RESULTS

The MRT detected reactions to 1- 4 cow's milk antigens in 20 out of 21 studied children (95.2%) (tab 1). The MRT showed positive reaction to all 4 milk fraction antigens in 6 (28.5%) patients; to 3 fractions in 19% , to 2 fractions in 7 children (33.3%)), and to 1 antigen in 3 children (14.2%). The highest percentage of positive reactions as determined by the MRT test was recorded to beta-lactoglobuline (80%) then to alpha-lactoalbumine (66%) followed by whey proteins (57.1%) and casein (47.6%) of the milk-allergic children.

**Table 1**. Resulrs of MRT Test among children with allergy to milk protein.

| Pat ID | Initials | Protein's of cow's milk | | | | Compliance in the range | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Casein | Beta-lactoglobuline | Alpha-lactoalbumine | Whey proteins | 4 | 3 | 2 | 1 | (-) |
| 1 | W.P. | + | + | + | + | | | | | |
| 2 | W.A. | - | + | - | + | | | | | |
| 3 | K.K. | + | - | | - | | | | | |
| 4 | Sz.A. | + | + | + | + | | | | | |
| 5 | Sz.F. | - | + | + | + | | | | | |
| 6 | G.B. | - | + | - | + | | | | | |
| 7 | B.S. | - | + | - | - | 6/2 | 4/2 | 7/2 | 3/2 | 1/2 |
| 8 | Z.E. | - | + | + | - | 1 | 1 | 1 | 1 | 1 |
| 9 | P.A. | - | - | + | - | | | | | |
| 10 | N.A. | - | - | - | - | | | | | |
| 11 | S.A. | + | + | + | - | | | | | |
| 12 | L.M. | + | + | - | - | | | | | |
| 13 | N.T. | + | + | + | + | | | | | |
| 14 | B.T. | - | + | - | - | | | | | |
| 15 | D.Mt. | - | + | - | + | | | | | |
| 16 | D.M. | - | + | + | + | | | | | |
| 17 | M.R. | - | + | + | + | | | | | |
| 18 | M.M. | + | + | + | + | | | | | |
| 19 | R.A. | + | + | + | + | | | | | |
| 20 | K.J. | + | + | + | + | | | | | |
| 21 | K.D. | + | - | + | - | | | | | |
| Positive results N % | | 0 | 17 | 14 | 12 | 3 | | | | |
| | | | 85.7 | 66.7 | 57.1 | | | | | |

In the control group 4 out of 6 patients demonstrated no positive reaction to any of four cow's milk fraction tested via MRT ( tab. 2) (66.6%). In two cases (33.3%) MRT tested positive to one of tested fraction: one beta-lactoglobuline and one whey protein, resulting in 16.6% false positive reaction to those milk fractions. There were no positive reactions neither to casein nor to alpha lactoalbumine in the control group.

**Table 2.** The results of MRT test in control group.

| Pat ID | Initials | | Proteins of cow's milk | | | Antigens compliance | |
|---|---|---|---|---|---|---|---|
| | | Casein | Beta-lactoglobuline | Alpha-lactoalbumine | Whey proteins | Negative to 4 antigens | Positive to 1 antigen |
| 1 | R.T. | | | | | | |
| 2 | K.M. | | | | | | |
| 3 | K.E. | | | | | | |
| 4 | B.R. | | | | | | |
| 5 | M.G. | | | | | | |
| 6 | S.D. | | | | | | |
| Positive results N% | | 0 | 1 | 0 | 1 | 66.6% | 33.3 |
| | | | 16.6% | | 16.6% | | |

Cell fractions reacting to cow's milk allergen in the studied children are idenitied in tab. #3.

**Table 3.** Type of cells that show positive reaction to cow's milk proteins in MRT test

| Type of cells | Positive reaction | |
|---|---|---|
| 1.Lymphocytes | 8 | 38.5 |
| 2.Granulocytes | 10 | 47.6 |
| 3.Lymphocytes, blood platelets | 3 | 14.2 |
| 4.All cells | 21 | 100 |

The highest percentage of positive reactions registered by the MRT test were observed in the granulocyte subpopulation 10/21(47.6%). Lymphocytes reacted in 8 cases (38.5%), and lymphocytes and blood platelets region was engaged in the reaction in 3 patients (14.2%). 2 fraction of proteins: beta-lactoglobuline and alpha lactoalbumine, were involved in the majority if all reactions. Tab. 4 shows the comparison of positive results of MRT test with the oral provocation trials( oral and lip) to cow's milk and results of skin test and the amounts of plasma IgE in the studied group.

**Table 4.** Results of other tests and MRT Test results among tested children.

| L.P. | Initials | Plasma IgE | Skin test with milk antigen | Provocative test with milk | | MRT | |
|---|---|---|---|---|---|---|---|
| | | | | Labial test | Oral test | Positive | # |
| 1 | W.P. | - | - | - | + | + | 4 |
| 2 | W.A. | 247 | (-) | - | + | + | 2 |
| 3 | K.R. | 11 | (-) | + | + | + | 2 |
| 4 | T.A. | 100 | (-) | + | + | + | 4 |
| 5 | S.L. | 19 | (-) | - | + | + | 3 |
| 6 | G.B. | 500 | (-) | - | + | + | 2 |
| 7 | B.S. | - | (+) | - | + | + | 1 |
| 8 | Z.E. | - | (-) | - | + | + | 2 |
| 9 | P.A. | 20 | (+) | - | + | + | 1 |
| 10 | N.A. | - | (-) | - | - | - | - |
| 11 | S.A. | - | (+) | - | + | + | 3 |
| 12 | L.M. | -(?) | (+) | + | not done | + | 2 |
| 13 | W.T. | - | (-) | not done | + | + | 4 |
| 14 | B.L. | - | (-) | + | + | + | 1 |
| 15 | K.M. | - | - | + | + | + | 2 |
| 16 | D.M. | - | - | + | + | + | 3 |
| 17 | M.R. | 269 | + | - | + | + | 3 |
| 18 | M.K. | 12.6 | (-) | - | + | + | 4 |
| 19 | K.A. | - | (+) | - | + | + | 4 |
| 20 | K.J. | 7 | (-) | - | + | + | 4 |
| 21 | K.D. | 42 | (+) | (+) | + | + | 2 |

The MRT method yield the sensitivity of 94.7% compared to the oral provocation. There were also a positive results of MRT tests among allergic children independent the level of plasma IgE ( correct and incorrect values), which were similar to the skin tests ( positive, negative) . These results indicate the probability that MRT test can detect reactions caused by harmful food in relation to studied population of blood cells independent of Type I Gell & Coombs ( reactions IgE dependent).

Tab.5 shows the results of studies on 30 patients allergic to cow's milk and hypersensitive to other food substances as tested by MRT and ALCAT using a 12 food antigen panel. The experimental group also included (7) children from the previously studied group with demonstrable hypersensitivity to cow's milk. 5 of them (83.3%) confirmed positive reaction to whole cow's milk.

The ability to identify reactions to specific food antigen was much higher in MRT test than in ALCAT ( tab.5).

**Table 5.** Comparison of ALCAT and MRT results.

| Antigen | Estimation number | Positive results Correlation vs clinical symptoms | | Total correlation | |
|---|---|---|---|---|---|
| | | MRT | ALCAT | MRT/ALCAT CORRELATION | % |
| Cod | 30 | 5 | 3 | 28/30 | 93.3 |
| Corn | 30 | 19 | 16 | 27/30 | 90 |
| Chicken | 30 | 5 | 3 | 28/30 | 93.3 |
| Eggs | 30 | 5 | 2 | 27/30 | 90 |
| Milk | 30 | 30 | 25 | 25/30 | 83.3 |
| Mushrooms | 30 | 6 | 4 | 28/30 | 93.3 |
| Pork | 30 | 11 | 10 | 29/30 | 96.6 |
| Potatoes | 30 | 6 | 4 | 28/30 | 93.3 |
| Rye | 30 | 4 | 2 | 28/30 | 93.3 |
| Tomatoe | 30 | 8 | 5 | 27/30 | 90 |
| Oat | 30 | 24 | 21 | 27/30 | 90 |
| Yeast | 30 | 9 | 7 | 28/30 | 93.3 |
| Total | 360 | 132 | 102 | 330/360 | 91.6 |
| Correlation | | 100% | 72.2% | 91.6% | |

## DISCUSSION

An allergy can develop as a result of different immunologic and non immunologic mechanisms (2,4,6,11,12,14,16-19). According to the classification of Gell-Coombs it is assumed that all pathogenic mechanisms can be engaged in any antigen reaction. Chandra (5) showed that among food sensitive patients, 48% of them showed clinical symptoms influenced by immediate mechanisms (IgE-dependent); and in 18% of patients cellular (lymphocyte, or Type-IV) reactions were observed. Reactions involving immune complexes responsible for clinical symptoms involved 10% patients; cytotoxic reactions constitute 6%; and mixed (involving more then one type of immunologic reaction ) are estimated to occur in 18%. As far as food intolerance is concerned, clinical symptoms can result if one pathogenic mechanism is involved. However, mixed reactions (immunologic and non-immunologic) should not be excluded, especially in chronic conditions (10). Given the presented theoretical evidence and, more often, clinical observations suggesting multiple pathogenic mechanisms, there is a need of laboratory documentation of these processes. The ALCAT method has elicited many questions and controversies because the spectrum of reactive evaluation was narrowed to the sub-population of granulocytes. It appears that a new generation test (MRT) expends the spectrum of total volumetric-change detection to granulocytes, lymphocytes and blood platelets. This makes it more reliable and diagnostically useful. In our study we used the MRT to confirm sensitivity to antigens of main fractions of cow's milk among milk sensitive children, with diagnosis confirmed by double blind placebo controlled oral challenge (10,11).

In only (1) case (4.8%) , out of 21 studied children, did the MRT Test provide a false negative result. The confirmation of lymphocytes 8/21-38.5%; lymphocytes and blood platelets 3/21-14.2% and granulocytes 10/21-47.6% playing roles in the pathogenic process is very valuable information. The results do not provide synonymous answers on what kind of reaction has been detected (immunologic, non-immunologic, cytotoxic) in vitro between antigen and the population of cells. However, it has been conformed that the MRT method detects both volumetric changes between the fractions of blood cells and plasma and registers and identifies the quantitative changes in their

limited population (lymphocytes, granulocytes and blood platelets). Therefore it expands the range of diagnostic information and suggests the pathogenic mechanism of existing changes. It is too preliminary, however, to make a determination of the diagnostic usefulness of the MRT test. Further multi-center studies as described in the Bindselev-Jensen publication (3) are needed to make a determination as to it's diagnostic application in food hypersensitivity.

**CONCLUSIONS**

1 The MRT appears to be useful in diagnosing states of food hypersensitivity in children allergic to cow's milk protein (it yields 94.7% sensitivity).

2 The results of MRT individual tests show qualitative-quantitative changes of different fractions of blood cells (lymphocytes, granulocytes and blood platelets) in reaction to studied (harmful) food antigen.

3 The MRT is more diagnostically reliable then ALCAT- but requires further multicenter studies to verify its clinical application in diagnosing food hypersensitivity.

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