CLINICAL INDICATIONS AND INTERPRETATION OF THE CAST

PC Potter, *MD, FCP(SA), FAAAAI, FACAAI*

Allergy Diagnostic & Clinical Research Unit, UCT Lung Institute, Observatory, South Africa

ABSTRACT

Non-IgE-mediated reactions to foods and drugs account for the majority of adverse reactions encountered in clinical practice. Until recently sensitivity to many environmental agents could only be confirmed by challenge tests, which are usually tedious and in some instances could be dangerous.

The cellular antigen stimulation test (CAST) is useful for detecting non-IgE-mediated sensitivity to food additives, preservatives and drugs. It can also confirm IgE-mediated sensitivity, but in general, specific IgE tests, such as skin-prick tests and the CAP RASTs are more efficient in this regard.

Cut-off values for the CAST for sulphites have been confirmed by double-blind placebo-controlled food challenges in the Allergy Diagnostic and Clinical Research Unit at the University of Cape Town. If selected by careful history taking CASTs can be a cost-effective diagnostic clinical tool.

Adverse reactions to food additives are occurring with increasing frequency in recent times, often manifesting as isolated episodes of angioedema and bronchospasm, but also as triggers of exacerbations in patients with chronic urticaria.

It is usually by careful history taking and keeping a diary of exposure in relation to clinical exacerbation that a dietary ingestant is identified as the possible culprit for the adverse reaction. Until recently, there was no laboratory test available to evaluate clinical sensitivity to non-IgE-mediated triggers of adverse reactions not only to food additives, but also to drugs, occupational antigens and substances in certain foods.

Allergy diagnostic tests based on the *in vitro* reaction of blood basophils to allergens have been of research interest for many years. However the basophil histamine release assay, although sensitive and reliable, is a tedious one and has been difficult to standardise. In recent years, however, the CAST-ELISA (Bühlmann Laboratories, Switzerland) has gained respectability as an important test in the allergy diagnostic arena,[1] if properly selected and interpreted. This article focuses on the principles of the cellular antigen stimulation test (CAST), the selection of patients and interpretation of the results.

PRINCIPLE OF THE CAST

The CAST depends on the exposure of interleukin 3 (IL-3) primed fresh basophils to different concentrations of an allergen, drug or chemical. Basophils which are sensitive to such exposure release sulphido leukotrienes into the media. These released leukotrienes are measured by an ELISA test. The CAST thus measures both IgE- and non-IgE-mediated leukotriene release in the ELISA.

Correspondence: Prof PC Potter, Allergy Diagnostic & Clinical Research Unit, UCT Lung Institute, PO Box 34560, Groote Schuur 7937, Cape Town, South Africa. Tel 021-406-6889, fax 021-406-6888, email ppotter@uctgsh1.uct.ac.za.

Cut-off values for non-specific leukotriene release have been determined by exposing (±20) healthy (non-allergic and non-sensitive) adult individuals to the agents, determining background release in this way. Patients who are clinically sensitive have leukotriene levels above the normal controls.

THE FLOW CAST

With developments in flow cytometry it is possible to measure upregulation of membrane markers of basophil activation (GP53 alias CD63) when basophils are exposed to allergens, drugs or other 'chemicals'.[2] This has resulted in the development of a flow-cytometric allergen stimulation test (FAST) now available as the Flow-CAST (Bühlmann Laboratories) or BASO Test (Becton-Dickinson).

The FAST may be used either utilising whole blood, or with leukocytes isolated by buffy coat centrifugation on sedimentation over dextran. The use of whole blood in this assay has the advantage of simpler manipulations (fewer centrifugation steps) but has lesser basophil recovery, interference with serum components, high activation in controls and interference by aggregated platelets also carrying CD63 markers and measured by FACS counting.[3]

Although the flow CAST is a sophisticated technique for measuring basophil activation upon allergen/chemical stimulation it requires expensive equipment (FACS machine) and highly trained laboratory technologists.

Thus the CAST ELISA has gained more widespread acceptance as a practically useful diagnostic tool, although the CAST ELISA is a highly specialised test to establish and maintain in the clinical laboratory. Some laboratories in Europe combine the CAST with the flow CAST in a single stimulation assay (CAST COMBI) to enhance sensitivity to the assay in evaluation of drug allergies.

THE CAST ASSAY

There are three procedural parts to the CAST assay.

Isolation of leukocytes

Dextran is added to the patient's blood in order to increase blood viscosity at 18-28˚C for 90 minutes after which the erythrocytes are sedimented. The supernatant containing the leukocytes is then transferred and subjected to a brief centrifugation to remove the thrombocytes and the pellet suspended in stimulation buffer containing IL-3.

Cell stimulation

Cells are stimulated for 40 minutes at 37˚C with an anti-IgE receptor antibody (stimulation control) or with no antibody (background) or 'allergen' in different concentrations. The supernatant is frozen or tested immediately for serum leukotriene (sLT) concentration in an ELISA.

Leukotriene determination

The ELISA is performed using precoated microtitre plates. Sixteen wells per assay are used for the stan-

dard curve and controls, 2 wells per patient for background, 2 wells per patient for stimulation control and 2 wells for each allergen. Enzyme label (alkaline phosphatase) and antibody are added to each well, incubated, and after a washing step, substrate solution (para-nitrophenyl-phospate) is added, incubated and stopped with 2N NaOH. Colour absorbance is measured at 405 nm in a microtitre plate reader.

Leukotriene release is reported in picograms(pg)/ml.

INTERPRETATION OF RESULTS OF THE ASSAY

For food and inhalation allergens, insect allergens and latex, Bühlmann have proposed that individuals with a net sLT stimulation yield higher than 200 pg/ml should be regarded as positive for the allergen tested.

Owing to the small increases of sLT with drug allergens, chemical allergens and food additives, Bühlmann have established an individual technical cut-off value for each allergen. These values represent the mean +3 standard deviations from up to 20 stimulated samples from normal blood donors.

The technical cut-off values are listed in Table I. Note that these are all above 40 pg/ml. It is important to understand that positive and negative predictive values for true clinical sensitivity for values above the technical cut-off values have not yet been determined. Thus the results of the CAST need to be carefully evaluated in relation to the clinical context of exposure in relation to reaction when found to be above or close to the technical cut-off value, to interpret the clinical significance of the result.

VALIDATION OF THE CLINICAL USEFULNESS OF THE CAST

In view of the uncertainties surrounding the significance of a previous cut-off value of 200 pg/ml for the sulphite CAST, our department undertook an evaluation of the CAST in a cohort of 20 patients who had suspected sensitivity to sulphites, comparing the result of the CAST with the result of a double-blind placebo-controlled food challenge (DBPCFC) in each of the subjects.[4]

Patients eliminated sulphites 48 hours prior to the challenge and then received 1, 5, 10, 15, 25, 50, 75, 100, 150 and 200 mg potassium metabisulphite diluted in preservative-free apple juice (30 ml) for each challenge. They received either a placebo or a sulphite challenge (24 hours apart). Sulphites were ingested at 10-minute intervals and vital signs (PEFR, pulse, BP and clinical symptoms) were monitored.

For this study, the significance of values specifically below 200 pg/ml (negative CASTs) which were obtained in 20 adult subjects previously clinically considered to be 'sulphite sensitive' (but not confirmed by the previous cut-off value) were studied.

Ten of 14 patients with 'negative CASTS' (below 200 pg/ml) had a positive challenge, while 6 patients with previous values above 200 pg/ml were also studied and 5 of these had a positive challenge.

Our laboratory found that sLT values above 40 pg/ml correlate extremely well with positive challenges to sulphites (83% overall). However, there are indeed subjects who are sensitive to sulphites whose basophils do release lesser amounts of sLTs on stimulation with sulphites *in vitro.* Fine tuning of the concentrations used in the assays or priming of the cells may improve this in the future.

RECOMMENDATIONS FOR USE OF THE CAST IN CLINICAL PRACTICE

1. The CAST is recommended as a useful test in the evaluation of non-IgE-mediated 'sensitivities' in clinical practice.
2. Although the CAST also reliably measures IgE-mediated sensitivities to inhalants, foods, insects and occupational allergens, the CAST is a more expensive test and not more sensitive than the CAP RAST for this indication. It is not recommended as a first-line test for IgE-mediated sensitivities in the South African context.
3. The CAST is most useful in the clinical evaluation of food additive and preservative sensitivity (e.g. sulphites, sodium benzoates and food colourants). The cut-off value for sulphite sensitivity (40 pg/ml) has been validated using DBPCFC at the Allergy Diagnostic and Clinical Research Unit of the University of Cape Town Lung Institute. For other additives the Bühlmann technical cut-off values should be interpreted in the clinical context.
4. In view of the poor sensitivity of specific IgE testing for drug allergy and non-steroidal anti-inflammatory drug sensitivity, the CAST ELISA or flow CAST may have a specific investigative application in this context. Studies by Sanz *et al.*[5] showed that combining the FAST with the CAST improved the sensitivity of the CAST, confirming sensitivity in 47% of cases who had positive skin tests to benzylpenicillin and amoxycillin and confirmed the specificity in 93% of patients who had negative skin tests and tolerated beta-lactams.
5. It has been suggested that the CAST may be useful in evaluating patients with clinical latex sensitivity who are skin test and RAST negative and the Allergy Diagnostic and Clinical Research Unit (ADCRU) is currently investigating this in a cohort of latex-sensitive health care workers.
6. A possible future application of the CAST is to measure the response of patients undergoing allergen immunotherapy. Preliminary data indicate that patients' basophils lose their sensitivity to allergen stimulation fairly early during allergen immunotherapy so this test may serve to identify responders and non-responders. This idea needs to be evaluated in prospective studies in the future.

REQUESTING A CAST

A limited number of laboratories conduct the CAST in South Africa. On selecting a CAST, a full history of specific exposure in relation to clinical symptoms should be provided to the laboratory in order to select the most likely 'allergen' in a cost-effective way.

A fresh sample of EDTA blood is required (2 x 4 ml specimens) and this should reach the laboratory in the morning on which the test is to be conducted, preferably within 3 hours of taking the blood sample. Patients should be off antihistamines and antileukotrienes for 48 hours prior to the test. It is also preferable to investigate patients 3 weeks after a severe adverse reaction.

We prefer the patient to be brought to the laboratory where blood is taken freshly and the patient can also be interviewed to assist intelligent selection of the most appropriate CAST reagent. It is important to ensure that patients are not on oral or injected steroids for 2 weeks prior to conducting a CAST on their basophils.

The result of a CAST is usually available within 24 hours and it is our policy to discuss each result with the

Table I. Technical cut-off values for CAST

Code	Allergen	Concentration in cell stimulation	Technical cut-off pg/ml sLT
Antibiotics			
BAG2-C1	Penicillin G	500 µg	50
BAG2-C2	Penicillin V	500 µg	40
BAG2-C11	PPL (benzylpenicilloylpolylysine)	5 µg	110
BAG2-C12	MDM (minor determinant mixture)	100 µg	100
BAG2-C203	Ampicillin	2 mg	70
BAG2-C204	Amoxicillin	200 µg	100
BAG2-C3	Cephalosporin C	20 µg	40
BAG2-C31	Cefamandole	500 µg	80
BAG2-C32	Cefazolin	500 µg	80
BAG2-C33	Cefuroxime	500 µg	40
BAG2-C61	Sulfamethoxazole	20 µg	50
BAG2-C62	Trimethoprim	20 µg	40
BAG2-C75	Tetracycline	20 µg	90
BAG2-C81	Ciprofloxacin	20 µg	90
Analgesics			
BAG2-C51	Lys-Aspirin	500 µg	90
BAG2-C52	Diclofenac	5 µg	40
BAG2-C53	Ibuprofen	20 µg	50
BAG2-C54	Indomethacin	2 µg	40
BAG2-C55	Acetaminophen	2 µg	60
BAG2-C56	Mefenamic acid	5 µg	60
BAG2-C57	Phenylbutazone	20 µg	80
BAG2-C58	Prophenazone	10 µg	40
BAG2-C59	Dipyrone / Metamizole	20 µg	50
Food Additives			
BAG2-C111	Sodium benzoate	500 µg	90
BAG2-C112	Sodium nitrate	20 µg	60
BAG2-C113	Potassium metabisulfite	10 µg	40
BAG2-C114	Sodium salicylate	200 µg	120
BAG2-C101	Food Colorant Mix I	20 µg	160
BAG2-C102	Food Colorant Mix II	5 µg	100
BAG2-C103	Tartrazine	1 mg	120
BAG2-CE104	Quinoline Yellow	100 µg	200
BAG2-CE110	Sunset Yellow Fcf	100 µg	40
BAG2-CE122	Chromotrope B	200 µg	80
BAG2-CE123	Amaranth	20 µg	40
BAG2-CE124	New Coccine	500 µg	100
BAG2-CE127	Erythrosine	1 µg	100
BAG2-CE131	Patent Blue V	50 µg	70
BAG2-CE132	Indigo Carmine	50 µg	40
BAG2-CE151	Brilliant Black Bn	50 µg	40
Anesthetics			
BAG2-CATR	Atracurium	1 mg	50
BAG2-CLID	Lidocaine	50 µg	40
BAG2-CMIV	Mivacurium	200 µg	40
BAG2-CPAN	Pancuronium	200 µg	110
BAG2-CPRO	Propofol	200 µg	100
BAG2-CROC	Rocuronium	200 µg	70
BAG2-CSUX	Suxamethonium	2 mg	40
BAG2-CTHI	Thiopental	100 µg	40
BAG2-CVEC	Vecuronium	50 µg	40
Environmental			
BAG-K79	Phtalic acid / anhydride	200 µg	40
BAG-K80	Formaldehyde	1 µg	40
BAG-K82	Latex		200
BAG-K85	Chloramine T	20 µg	90
BAG-K87	A-Amylase	20 µg	200

patient and to provide specific written information to facilitate avoidance of the allergen/preservative/additive/drug to which the patient is found to be sensitive.

Declaration of conflict of interest

The author has no conflict of interest as he received no personal remuneration or conference funding. Buhlmann Laboratories (Basel, Switzerland) funded the sulphite studies conducted on the CAST test at the UCT Lung Institute (by way of provision of reagents and contributing to the technologist's salary).

REFERENCES

1. De Weck AL, Stadler BM, Urwyler A, Wehner HH, Bulman RP. Cellular Allergen Stimulation Test (CAST). *Allergy Clin Immunol* New 1993; **5**: 9-14.
2. Sainte-Laudy J. Application of flow cytometry to the analysis of activation of human basophils: Immunological validation of the method. *Allerg Immunol* 1998; **30**: 41-43.
3. De Weck A, Sanz M. Flow cytometric cellular allergen stimulation test (FAST/flow CAST): Technical and clinical evaluation of a new diagnostic test in allergy and pseudo allergy. *ACI International* 2002; **14**: 204-215.
4. Hawarden D, Fenemore B, Hill L, *et al.* Evaluation of the cellular antigen stimulation test (CAST) vs DBPCFC in a cohort of patients with suspected adverse reactions to sulphites (Poster). *Current Allergy & Clinical Immunology* 2004; **17**: 138.
5. Sanz M, Gamboa PM, de Weck AL. Clinical evaluation of *in vitro* tests in diagnosis of immediate allergic reactions to B lactam antibiotics. *ACI International* 2002; **14**: 185-193.