IS SPECIFIC ANTIBODY DETERMINATION DIAGNOSTIC FOR ASTHMA ATTRIBUTABLE TO LOW-MOLECULAR-WEIGHT AGENTS?

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Reprinted from **OCCUPATIONAL MEDICINE: State of the Art Reviews**
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210 S. 13th St., Philadelphia, PA 19107 (215) 546-7293

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This chapter reviews information regarding the use of specific antibody determination in the diagnosis of asthma induced by low-molecular-weight (LMW) agents. Methods of specific antibody determination considered include *in vitro* methods such as radioallergosorbent test (RAST) or enzyme-linked immunosorbent test (ELISA), and *in vivo* methods such as allergen skin testing. LMW agents are defined as those compounds with a molecular weight < 5000 daltons.[11,39]

ANALYSIS OF TEST PERFORMANCE

No single test can be relied upon to rule out or diagnose a medical condition with complete accuracy. It is therefore necessary to formally assess the performance of a test and validate its ability to discriminate between the presence and absence of disease to determine its place in the diagnostic armamentarium. Statistical terms used to describe test performance include sensitivity, specificity, positive predictive value, and negative predictive value (Table 1). **Sensitivity** is the probability that the diagnostic test is positive in individuals with the disease. **Specificity** is the probability that the diagnostic test is negative in individuals who do not have the disease. **Positive predictive value** is the probability that an individual with a positive test result has the disease. **Negative predictive value** is the probability that an individual with a negative test does not have the disease.

TABLE 1. Definitions of Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value

	Test Result	
Disease	*Negative*	*Positive*
Absent	A	B
Present	C	D

Sensitivity = D/C + D), Specificity = A/(A + B), Positive predictive value = D/(B + D), Negative predictive value = A/(A + C)

Documentation of these performance characteristics is critical in determining how a diagnostic test is interpreted. For example, the choice of a threshold value used to discriminate between a positive and negative test can markedly affect sensitivity and specificity. In the case of specific antibodies to LMW agents, a high threshold value might improve specificity but decrease sensitivity, and a lower threshold value would have the opposite effect. The threshold value chosen should be one which optimizes sensitivity and specificity. Receiver operating characteristic (ROC) curve analysis is a formal method for identifying such optimal threshold values.

Performance characteristics of a test in predicting the presence of a condition are related not only to the test itself, but also to the prevalence of the condition in the population studied. For example, if a condition is present at low prevalence, number "A" in Table 1 will be markedly greater than number "C." This will result in a high negative predictive value (NPV) that may be more related to the low prevalence of the condition in the population than the ability of the test to rule out the condition. Another effect of low prevalence of a condition is that even a relatively low rate of false positive tests can result in number "B" in Table 1 being large in relation to number "D," resulting in the test having low positive predictive value (PPV) for the condition in that population.

Documentation of test performance impacts on how a test is used. For example, a test that was very sensitive but had only a moderate PPV in the population being tested might be used to screen for a condition, but individuals with a positive test would require additional evaluation before a definitive diagnosis could be made. A test with low sensitivity but high PPV might not be useful for screening, but could be clinically useful for confirming the presence of disease. A test with high NPV could be used to help rule out a disease.

Although data allowing analysis of the relationship between presence of specific antibodies to LMW chemicals and asthma caused by these agents has been collected in research settings and is described in this review (Table 2), results may not always be applicable to assays that are commercially available. Although similar in principal, commercially available assays may use substantially different reagents and procedures from those documented by research units. Use of unstandardized solid-phase allergens in these tests is a particular problem.[12] It is therefore important that the practitioner be aware that performance characteristics for tests as done in different laboratories are not necessarily similar.

THEORETICAL PROBLEMS IN DIAGNOSING ASTHMA INDUCED BY LMW AGENTS THROUGH DEMONSTRATION OF SPECIFIC ANTIBODIES

Although tests demonstrating the presence of specific antibodies to LMW agents may have an important adjunctive role in the diagnosis of asthma induced by

TABLE 2. Studies Evaluating Performance of Tests for Specific Antibodies to LMW Agents in Predicting the Presence of Asthma and Related Conditions.*

Agent	Test	Population	Health Effect	Sensitivity	Specificity	PPV	NPV	Reference
TMA	TMA-HSA IgE ELISA	Cross-sectional	TMA-induced asthma	100%	93%	19%	100%	17
TMA	TMA-HSA IgE ELISA	Longitudinal	TMA-induced asthma	90%	94%	56%	99%	17
Acid anhydrides	TMA-HSA, PA-HSA, maleic acid-HSA skin tests	Historical prospective cohort	Symptoms	30%	99%	83%	93%	5
Platinum salts	Prick skin test	Historical prospective cohort	Symptoms	46%	100%	100%	58%	38
Platinum salts	Prick skin test	Cross-sectional	(+) cold air challenge	45%	90%	33%	93%	2
Platinum salts	Prick skin test	Cross-sectional	(+) cold air challenge	47%	87%	39%	90%	8
Platinum salts	Prick skin test	Suspected disease	(+) methacholine challenge	71%	33%	89%	13%	24
Platinum salts	Prick skin test	Suspected disease	(+) platinum salt challenge	82%	80%	95%	50%	24
Various isocyanates	Isocyanate-HSA IgE ELISA	Suspected disease	(+) isocyanate challenge	31%	97%	90%	62%	10
Various isocyanates	Isocyanate-HSA IgG ELISA	Suspected disease	(+) isocyanate challenge	72%	76%	72%	76%	10
Isocyanates (TDI)	TDI-HSA IgE RAST	Suspected disease	(+) TDI challenge	3%	97%	50%	46%	19
Isocyanates (TDI)	TDI-HSA IgG RAST	Suspected disease	(+) TDI challenge	3%	97%	50%	46%	19
Various isocyanates	Isocyanate-HSA IgE RAST, Class II/III	Suspected disease	isocyanate-induced asthma	28% (Class II) 19% (Class III)	91% (Class II) 98% (Class III)	80% (Class II) 92% (Class III)	48% (Class II) 47% (Class III)	34
Isocyanates (TDI)	TDI-HSA IgE ELISA	Suspected disease	(+) TDI challenge	14%	92%	N/A	N/A	26
Isocyanates (TDI)	TDI-HSA IgG ELISA	Suspected disease	(+) TDI challenge	46%	92%	N/A	N/A	26
Western red cedar	Plicatic acid-HSA IgE RAST	Suspected disease	(+) plicatic acid challenge	44%	100%	100%	50%	35
Western red cedar	Plicatic acid-HSA IgE RAST	Suspected disease	(+) plicatic acid challenge	30%	ND	ND	ND	20
Western red cedar	Plicatic acid-HSA IgE RAST	Cross-sectional	(+) methacholine challenge	17%	95%	46%	82%	36
Western red cedar	Plicatic acid-HSA IgE RAST	Longitudinal	(+) methacholine challenge	18%	94%	59%	71%	37

* Sensitivity, specificity, PPV, and NPV calculated from data provided in reference.
HSA = human serum albumin, LMW = low moledular weight, NPV = negative predictive value, PA = phthalic anhydride, PPV = positive predictive value, TDI = toluene diisocyanate, TMA = trimellitic anhydride.

these agents, a number of factors adversely impact on the performance characteristics of such tests. Even in asthma induced by production of IgE to well-characterized high molecular weight (HMW) agents such as house dust mite, demonstration of specific circulating IgE by RAST or skin test does not perfectly predict the presence of asthma or response to specific inhalation challenge with allergen.[31,32] Factors related to host response, as well as technical factors affecting test performance, might account for suboptimal test performance in predicting asthma induced by LMW agents.

From the standpoint of the host response, at least four factors are relevant. **First**, although there is a relationship between the presence of specific IgE to an agent and asthma induced by that agent, atopy (the tendency to produce IgE) and other characteristics underlying the asthmatic phenotype (such as airways hyperreactivity) are under different genetic control.[27] Thus, individuals with specific IgE to an agent may have asthma, a clinical manifestation other than asthma (such as rhinitis), or even no symptoms at all. **Second**, the assumption that antibodies demonstrated to be present in skin or blood are also present in the airways may not always be correct. **Third**, for many LMW agents, the primary mechanism for induction of asthma may not even be through induction of IgE responses but rather by other as-yet undetermined mechanisms.[11]

Fourth, specific antibodies to LMW agents may be present without disease, suggesting that such antibodies can simply be biomarkers of exposure to these agents. For example, in a recent study of workers exposed to 4,4'-diphenylmethane diisocyanate (MDI), no cases of occupational asthma were documented, yet exposed workers had significantly greater levels of MDI-specific IgG demonstrated in blood than was present in workers not exposed to MDI.[23] Similarly, a study of car painters exposed to vapors and aerosols of paints containing prepolymer and monomer of hexamethylene diisocyanate (HDI) demonstrated a relationship between exposure and the presence of serum HDI-specific IgG, but not a relationship between respiratory symptoms and specific antibodies.[40]

An important technical problem in detecting specific antibodies, either by skin testing or through the use of *in vitro* methods, is that antigens used for the detection of antibodies may lack clinically relevant antigenic determinants or contain antigenic determinants irrelevant to the disease of interest. This is a particular problem for LMW agents, where test antigens for the detection of specific antibodies are likely not to optimally parallel those generated *in vivo* by inhalation exposure.[12] In general, LMW agents induce antibody responses by acting as haptens, combining with target body proteins to form complete antigen capable of inducing these responses.[39] Immunogenic epitopes expressed by such conjugates may either be related to the LMW agent itself, or the result of new antigenic determinants generated by interactions specific to that particular combination of hapten and carrier protein.[1,16,22,42] Although most work has used human serum albumin (HSA) as the carrier protein to which the LMW agent is coupled for use in immunoassays, actual *in vivo* target proteins for binding by the LMW agent after inhalation exposure are likely to be proteins present in the airway, such as those associated with airway epithelial cells.[30] Using inappropriate hapten-carrier protein complexes as test antigens in immunoassays for LMW agent–specific antibodies could, depending on the individual case, result either in failure to detect antibodies relevant to the worker's respiratory disease, or detection of antibodies irrelevant to the worker's respiratory disease.

Finally, it should be recognized that performance of tests for specific antibodies in the diagnosis of asthma induced by LMW agents may differ greatly among agents

(Table 2). For example, it is well recognized that some LMW agents such as acid anhydrides and platinum salts often induce occupational asthma by IgE-dependent mechanisms. For other agents, such as isocyanates, specific IgE is either not found or found in only a minority of patients.[11] It would be expected that test performance would be better for the former agents, and worse for the latter agents.

SPECIFIC LOW-MOLECULAR-WEIGHT AGENTS

Acid Anhydrides

The acid anhydrides are a group of reactive, LMW chemicals widely used as curing agents in the manufacture of epoxy and alkyd resins, which form the base for paints, varnishes, and plastics.[41,44] Anhydrides causing asthma include phthalic anhydride (PA), used in the manufacture of plastics; trimellitic anhydride (TMA), used in the production of epoxy resins, plastics, and paints; hexahydrophthalic anhydride, used in electrical transformer production; himic anhydride, used in fire retardant production; and tetrachlorophthalic anhydride (TCPA), used in epoxy resin production. It has long been suspected that specific antibody plays a significant role in induction of asthma by these agents. In the case of phthalic anhydride, a study as early as 1939 suggested a pathogenic role for reaginic antibody.[44] A more recent study documented the ability of serum from a worker with TMA asthma to passively transfer specific bronchial reactivity to TMA to rhesus monkeys.[13] An association of HLA-DR3 with formation of specific IgE against TMA, but not PA, has been demonstrated in acid anhydride workers.[41]

TMA exposure has been reported to be capable of inducing several different types of immune-mediated respiratory diseases. Immediate-type asthma has been well documented to be mediated by IgE with specificity for TMA-HSA conjugates.[17,43] Other syndromes associated with the presence of IgG able to be bound by TMA-HSA conjugates include the late respiratory systemic syndrome, a disease similar to hypersensitivity pneumonitis; late asthma, an airway response occurring 6 to 12 hours after TMA exposure; and pulmonary disease anemia syndrome, which is characterized by dyspnea, hemoptysis, pulmonary infiltrates, and anemia.

Specific IgE responses to TCPA were found to be associated with cases of occupational asthma in workers exposed to epoxy resin powder containing TCPA that was used to encapsulate electronic components in a protective plastic coat.[18] Although not a population study, this report documented positive IgE RASTs and skin prick tests to TCPA-HSA in all 7 of 7 patients referred from a plant to be evaluated for occupational asthma. Occupational asthma was confirmed clinically in 3 patients, and by specific inhalation challenge with epoxy resin dust and pure TCPA in 4 patients. Mean TCPA-IgE RAST levels were greater in patients than in a group of exposed controls.

Subsequent findings in 6 of these 7 patients over 12 years of follow-up was recently reported (one died shortly after diagnosis).[4] TCPA-IgE RAST levels fell rapidly over the first several years after removal from exposure, but more slowly after that. A log-log model described the decline in TCPA-IgE RAST levels over time with an r^2 of 0.94. TCPA-IgE RAST skin tests remained positive in all tested subjects at 8 years after removal from exposure, but were positive in only 3 of 5 subjects after 12 years.

In contrast to findings supporting a role for detection of IgE-specific antibodies in the diagnosis of TCPA-induced asthma, a cross-sectional survey of a plant where epoxy resin containing TCPA was used to manufacture solenoid coils did not find a

relationship between the presence of TCPA-HSA-specific IgE by ELISA and the presence of respiratory symptoms.[21] However, a large proportion of workers (31%) had elevated serum specific IgE by TCPA-HSA ELISA which was related to level of exposure.

A recently reported historical cohort study provides data allowing calculation of the performance of acid anhydride (AA)-HSA skin tests in predicting work-related respiratory symptoms (assessed by questionnaire).[5] Workers were recruited from four factories where acid anhydrides were used, including PA, TMA, and maleic acid. The cohort was drawn from all workers who had started to work in an area where acid anhydrides where used and continued to work there for > 1 month. In two of the plants, the retrospective period evaluated was 32 years, while in the other two plants the period was 13 years. Out of a target population of 506 workers, data are presented relating symptoms and AA-HSA skin test results for 366 workers. Many had left work by the time of evaluation, but duration away from work, which might have affected sensitivity,[4] is not documented. Nine percent (33/366) reported new work-related respiratory symptoms; 3% (12/366) had positive AA-HSA skin tests. Eight of the 12 workers with positive skin tests came from a single factory where only TMA was used. AA-HSA skin tests predicted work-related respiratory symptoms with a sensitivity of 30%, specificity 99%, PPV 83%, and NPV 93%.

A recent study of workers involved in the manufacture of TMA documents the performance characteristics of an ELISA detecting serum IgE specific for TMA-HSA conjugate in evaluating the presence of occupational asthma caused by TMA.[17] A cross-sectional survey of 181 workers in 1990 lead to clinical documentation of asthma (apparently based on interview by the plant nurse, but details are not provided) in 3 workers, and a positive ELISA for TMA-specific IgE in 16 workers. The sensitivity of the assay in detecting prevalent TMA-asthma was 100%, the specificity 93%, the PPV of the assay 19%, and the NPV of the assay 100% (figures presented here differ from the original report, in which the table presenting this data is mislabeled). Of these subjects, 119 were followed over the next 5 years. These subjects included all 16 with a positive ELISA for TMA-specific IgE at baseline; within 3 years an additional 6 of these workers developed asthma, bringing the total to 9. Only 1 of 103 workers without TMA-specific IgE developed asthma during this time period. Overall, the sensitivity of the ELISA for TMA-specific IgE in predicting asthma over the next 5 years was 90%, specificity 94%, PPV 56%, and NPV 99%.

Thus, although numbers of cases were relatively small, the evaluated assay for TMA-specific IgE had high sensitivity for prevalent and incident TMA-induced occupational asthma in TMA-exposed workers. The PPV was not as good, so workers positive for TMA-specific IgE would require additional evaluation before TMA-induced asthma could be diagnosed. However, in view of the high sensitivity of the assay, it appears to have potential for use as a screening tool in TMA-exposed workers. However, data are limited, so firm recommendations about the utility of assessment for TMA-HSA-specific IgE cannot be made at this time. Finally, in view of extremely limited data, the role of specific IgE determination in diagnosis of asthma caused by other acid anhydrides also remains to be defined.

Platinum Salts

Platinum salts are well known for their ability to induce IgE-sensitization and IgE-mediated allergic disease in exposed workers.[2,8,9,24,25] Occupational asthma,

rhinitis, conjunctivitis, and eczema all have been described in workplaces associated with platinum exposure, including platinum refineries and catalyst production plants. Risk of IgE sensitization is strongly related to cigarette smoking and is highly associated with the presence of symptoms compatible with platinum salt allergy.[9,38]

IgE-sensitization to platinum salts can be demonstrated by skin prick test, circumventing problems associated with *in vitro* tests for platinum-specific IgE. Although *in vitro* tests can be performed, reported performance relative to prick skin test has been variable. For example, in one report only 23 of 38 workers with positive platinum salt skin tests identified in a cross-sectional survey of South African platinum refinery workers also had positive RASTs to platinum-HSA. Furthermore, 16 of 268 skin test negative workers also had positive RASTs.[25] By contrast, in a survey of a platinum refinery in the U.S., 20 of 22 platinum skin test positive subjects also had elevated levels of platinum-specific IgE demonstrated by RAST. However, 8 of 94 skin test negative workers also had positive RASTs.[8]

Performance of platinum skin prick tests in discriminating between those with and without symptoms,[38] positive reactions to cold air challenge,[2,8] methacholine challenge,[24] and specific inhalation challenge using platinum salt[24] all have been described in sufficient detail to calculate sensitivity, specificity, PPV, and NPV. One report is a historical prospective cohort study from which relationships between platinum test results and "symptoms" (predominantly respiratory symptoms and clinical diagnoses of asthma derived from plant medical records) can be calculated.[38] In this study, a cohort was defined as workers entering employment over a 2-year period as chemical process operators in platinum group metals refineries. They were followed either until leaving refinery work, or until the end of the study 7 years later, whichever came first. Skin prick tests with platinum salts were carried out on all refinery workers every 3 to 6 months. Out of a potential group of 112 workers identified, data for skin tests and symptoms are available to allow calculation of test performance for 84 workers: 26% (22/84) developed positive skin tests; 57% (48/84) developed symptoms. Platinum salt skin testing predicted symptoms with a sensitivity of 46%, specificity 100%, PPV 100%, and NPV 58%.

Two reports[2,8] detail cross-sectional evaluation of platinum refinery workers in the U.S. with platinum salt skin testing and assessment of nonspecific bronchial hyperreactivity by cold air challenge. Because of a high rate of termination from employment due to suspected platinum salt sensitivity, both current and terminated employees (average duration since termination from employment of 5 years) were evaluated. Considering only current employees,[2] platinum skin test predicted a positive response to cold air challenge with a sensitivity of 45%, a specificity of 90%, a PPV of 33%, and a NPV of 93%. Considering both current and terminated employees,[8] test performance in predicting a positive response to cold air challenge was similar: sensitivity 47%, specificity 87%, PPV 39%, and NPV 90%. The investigators also reported similar sensitivity and specificity of the platinum skin test in identifying workers with asthma symptoms of 35% and 69%, respectively.

Interestingly, in a follow-up evaluation performed one year later, 3 of 4 workers with a positive cold air challenge but a negative platinum salt skin test converted their skin test to positive. In contrast, only two of 63 workers with negative cold air challenges and negative skin tests converted their skin tests to positive. Thus, in some cases, nonspecific airways hyperreactivity preceded the development of platinum-specific IgE demonstrable by skin test.[8]

A study of German platinum refinery workers[24] assessed responses to platinum salt skin testing, nonspecific bronchial hyperreactivity by methacholine challenge,

and antigen-specific reactivity to bronchial provocation tests with platinum salts. The study population was quite different from that of the previously-cited U.S. studies[2,8] in that evaluated platinum workers were all symptomatic individuals referred to a pulmonary department by the Ministry of Social Welfare. A total of 35 such symptomatic workers were referred over the 7 years between February, 1983 and February, 1990. Twenty-seven underwent platinum salt skin testing, methacholine challenge, and bronchial provocation with platinum salts. In this population, platinum salt skin testing predicted nonspecific bronchial hyperreactivity to methacholine with a sensitivity of 71%, specificity 33%, PPV 89%, and NPV 13%. Performance in prediction of a positive response to bronchoprovocation with platinum salts was somewhat better, with a sensitivity of 82%, specificity 80%, PPV 95%, and NPV 50%.

Thus, due to low sensitivity in predicting symptoms or bronchial hyperreactivity, platinum salt skin testing alone does not appear to have optimal performance characteristics for use in screening for asthma in platinum refinery workers.[2,8,38] PPV in platinum refinery workers also has been variable, so the implications of a positive test in this population are not completely clear. In view of moderate sensitivity and high PPV in predicting the outcome of bronchial challenge with platinum salts, platinum salt skin testing does appear to be potentially useful in confirming the presence of platinum-induced occupational asthma in symptomatic workers where there is a high clinical index of suspicion.[24] However, NPV is poor, so a negative skin test would not rule out platinum-induced occupational asthma.

Isocyanates

Isocyanates are highly reactive chemicals used in the production of polyurethane foams, elastomers, adhesives, varnishes, coatings, and paint hardeners. In the U.S., it is estimated that over 100,000 workers are exposed to isocyanates each year. These chemicals are both irritative and among the most common inducers of occupational asthma.[3,6,28,33] The three most commonly used diisocyanates include toluene diisocyanate (TDI), 4,4'-diphenylmethane diisocyanate (MDI), and hexamethylene diisocyanate (HDI). In applications such as painting, use of MDI and HDI has become far more common in recent years, as they have lower vapor pressures than TDI.

Problems in the use of specific antibody responses to predict isocyanate-induced asthma were noted previously and include induction of antibodies simply by exposure, rather than disease,[23,40] problems in generating appropriate antigens for use in assays,[12,30] and the issue that isocyanates may not induce asthma primarily via an antibody-mediated mechanism.[3,6,11,28]

Current evidence suggests that generation of specific IgE does not underlie the bulk of isocyanate-induced asthma, in that only 10–30% of symptomatic isocyanate workers have detectable levels of circulating IgE antibodies to diisocyanate-HSA conjugates.[28] Other immunologic mechanisms, such as T-cell–mediated processes, may more frequently underlie isocyanate asthma.[3,6,28] Recent data such as infiltration of the airway with T cells after isocyanate challenge in sensitive workers[29] and probable increased usage of Vβ1 and Vβ5 variable β gene segments in T-cell receptors of isocyanate-specific T cells[7] support the likely important role of T cells in mediating isocyanate asthma. If not linked to pathogenesis, presence of specific antibodies might not predict T-cell–mediated asthma.

Despite these theoretical problems, several studies have evaluated the ability of isocyanate-specific antibody determination to predict positive responses to isocyanate

challenge in symptomatic workers felt likely to have isocyanate asthma.[10,19,34] In the study of Cartier et al., subjects were workers referred for evaluation with specific inhalation challenge in a tertiary care center.[10] Subjects had been exposed at work to TDI, HDI, or MDI. Isocyanate-specific IgE and IgG were measured in serum by ELISA using the appropriate isocyanate-HSA conjugate as solid phase antigen. Bronchial challenge was carried out using the appropriate isocyanate. Tests for isocyanate-specific IgE were predictive for a positive specific bronchial challenge with a sensitivity of 31%, specificity of 97%, PPV of 90%, and NPV of 62%. Tests for isocyanate-specific IgG had a sensitivity of 72%, specificity 76%, PPV 72%, and NPV 76%. Of note is that all individuals positive for specific IgE also were positive for specific IgG. In a follow-up study using sera from these same individuals[16] isocyanate-specific IgE and IgG were measured by ELISA, with results expressed as an index of color development relative to negative control sera. Mean ELISA IgG-index was greater in workers with positive bronchial isocyanate challenges than in workers with negative challenges. A similar trend was noted for ELISA IgE-index. Unfortunately, numbers of positive and negative tests in the challenge-positive and challenge-negative groups are not specifically stated, so sensitivity, specificity, PPV, and NPV of the reported ELISA cannot be calculated.

The study of Karol et al.[19] evaluated individuals felt to be sensitized to TDI. IgE determination was by RAST, and IgG determination was by ELISA, in both cases using TDI-HSA as the antigen bound to solid phase. Subjects also underwent specific bronchial challenge with TDI. Of a total of 63 subjects, 34 had positive responses to TDI bronchial challenge, 2 were positive for isocyanate-specific IgE, and 2 were positive for specific IgG. No subjects were positive for both IgE and IgG. For both IgE and IgG, one positive subject had a positive bronchial challenge with TDI, and one did not. Thus, determination of isocyanate-specific IgE predicted positive response to TDI bronchial challenge had a sensitivity of 3%, specificity 97%, PPV 50%, and NPV 46%. Performance characteristics for determination of isocyanate-specific IgG were identical.

The study of Tee et al.[34] also evaluated symptomatic workers referred for evaluation at a tertiary care center. Workers had been exposed to a variety of isocyanates. Antibody determination was performed using a commercially-available RAST system (Phadebas RAST, Pharmacia and Upjohn Ltd., Uppsala, Sweden) with RASTs performed to TDI-HSA, MDI-HSA, and HDI-HSA. Objective documentation of isocyanate-induced occupational asthma was either by bronchial challenge using the appropriate isocyanate, or ambulatory peak flow monitoring at and away from work. Performance characteristics of isocyanate-specific IgE determination in serum to predict a diagnosis of isocyanate-induced occupational asthma were determined using a lower (Class II) and a higher (Class III) threshold value to define RAST positivity. Using the Class II threshold, sensitivity was 28%, specificity 91%, PPV 80%, and NPV 48%. Using the Class III threshold, sensitivity was 19%, specificity 98%, PPV 92%, and NPV 47%.

Park et al.[26] evaluated the prevalence of specific IgE and IgG antibodies to TDI-HSA as determined by ELISA in symptomatic TDI workers from the same work place with ($n = 50$) and without ($n = 13$) positive responses to bronchial provocation with TDI. It is unclear whether the study subjects represent a random sample of symptomatic workers. Thus, sensitivity and specificity for the ability of antibody testing to predict response of symptomatic workers to specific bronchial challenge can be calculated from this study, but PPV and NPV cannot. TDI-HSA IgE ELISA predicted response to TDI bronchial challenge with a sensitivity of 14% and a specificity

of 92%. TDI-HSA IgG predicted response to TDI bronchial challenge with a sensitivity of 46% and a specificity of 92%.

Thus, studies in symptomatic isocyanate-exposed individuals in which there is a high clinical index of suspicion for isocyanate-induced occupational asthma suggest that tests for isocyanate-specific IgE have low sensitivity for the condition. Two studies[10,34] suggest that, despite low sensitivity, the test has good PPV; and one study[19] suggests poor PPV. Thus, due to poor sensitivity, testing for isocyanate-specific IgE does not currently appear to have promising performance characteristics for use as a screening tool for isocyanate-induced occupational asthma. It may still have usefulness as a confirmatory test for individuals in whom isocyanate-induced occupational asthma is suspected, but due to conflicting published data this issue remains to be resolved.

Several studies demonstrate that isocyanate-specific IgG can be detected in exposed individuals in the absence of asthma.[23,40] Thus, determination of isocyanate-specific IgG would appear to have potential usefulness as a biomarker of exposure, but its precise role remains to be determined.

Western Red Cedar (Plicatic Acid)

Occupational asthma caused by western red cedar (WRC; *Thuja plicata*) is a common problem in sawmill industries of the North American Pacific Northwest and has been studied extensively by investigators at the University of British Columbia, Vancouver, Canada.[14,15,20,35,36,37] Asthma occurs in approximately 5% of exposed workers and is thought to result from sensitization to plicatic acid (PA) a LMW organic compound which constitutes 40% wt/wt of cedar dust. Controlled bronchial challenge with PA induces objective asthmatic responses in individuals with WRC asthma, including immediate, late, and dual reactions.

Only a minority of individuals with WRC asthma have demonstrable serum IgE with specificity for PA-HSA conjugate.[20,35] Similar considerations as those noted for isocyanates might explain this finding. As is the case for other LMW agents, it is not clear that conjugation to HSA provides the optimal antigen for use in immunoassays. In addition, it is unclear what proportion of individuals with WRC asthma, if any, have disease that is mediated by IgE. In this regard, one report documented that PA was able to cause basophil histamine release in peripheral blood cells from subjects with WRC asthma but did not induce basophil histamine release by cells from controls. However, the histamine release did not appear to be mediated by IgE.[14]

As is the case for isocyanates, mediation of WRC asthma by T cells also has been suggested as an explanation for lack of PA-specific IgE in many WRC asthma patients. A report has documented significant proliferative responses of peripheral blood mononuclear cells to PA-HSA (defined as a stimulation index of greater than 2) in 24% (8/33) of WRC asthma patients, but in none of 10 exposed nonasthmatic workers and only one of 18 nonasthmatic controls.[15] Although the proportion of individuals with detectable serum IgE to PA-HSA was not reported, it was suggested that in at least some of these patients, WRC asthma might be mediated by T cells.

Several studies provide data that can be used to estimate performance characteristics of PA-HSA RAST for specific IgE in predicting the presence of WRC asthma.[20,35,36,37] The study of Tse et al. assessed for specific IgE against PA-HSA in the sera of 28 workers suspected to have WRC asthma and referred for evaluation by their personal physicians.[35] In 18 of these workers, the diagnosis was confirmed by bronchial challenge with PA. Ten workers did not react to bronchial challenge with PA. Eight of the 18 workers with diagnostic bronchial challenges, and none of the 10

workers with negative bronchial challenges, were found to have elevated PA-HSA RAST values. Thus, in this study, PA-HSA RAST predicted a positive bronchial challenge response to PA with a sensitivity of 44%, specificity 100%, PPV 100%, and NPV 50%.

The study of Lam et al. confirmed poor sensitivity of PA-HSA IgE RAST in predicting positive bronchial challenge with PA in symptomatic red cedar workers.[20] In this study, PA-HSA IgE was assessed only in subjects with WRC asthma confirmed by bronchial challenge with PA. Of such workers, only 13 of 44 were noted to have significantly elevated RASTs, for a sensitivity of only 29.5%. Since only individuals with positive PA challenges were studied, specificity, PPV, and NPV cannot be determined from this report.

A large cross-sectional study of a single sawmill conducted by Vedal et al. allows estimation of the ability of PA-HSA RAST to predict positive responses to methacholine challenge in red-cedar sawmill workers.[36] Unfortunately, data used to make these calculations are derived from only 477 of the 625 workers who had PA-HSA RAST performed, as 148 workers did not undergo methacholine challenge, at least in part due to exclusion criteria. However, the prevalence of RAST positivity in workers not undergoing methacholine challenge (5%) was not significantly different from that in workers undergoing methacholine challenge (7%). Using data from the 477 workers undergoing both procedures, the PA-HSA RAST predicted positive methacholine challenge results with a sensitivity of only 17%, specificity 95%, PPV 46%, and NPV 82%. Additional findings of note were that neither PA-HSA RAST positivity nor hyperresponsiveness to methacholine were associated with atopy, and that, independent of its association with bronchial hyperreactivity, RAST positivity was not associated with asthma.

Vedal et al. followed the population noted above longitudinally over a 2-year period and reported results that can be used to calculate the ability of PA-HSA RAST to predict positive responses to methacholine challenge in red-cedar sawmill workers during the subsequent 2 years.[37] Unfortunately, due to large layoffs at the sawmill, only 227 workers are identified as having both determination of PA-HSA RAST and at least two methacholine challenges during three surveys over the 2-year period. PA-HSA RAST positivity predicted positive methacholine challenge at any survey with a sensitivity of 18%, specificity 94%, PPV 59%, and NPV 71%.

In summary, currently available data suggests that measurement of specific IgE by PA-HSA RAST has poor sensitivity for the detection of WRC asthma. Thus, measurement of specific IgE by PA-HSA RAST does *not* appear to have promise for use in medical screening of red cedar workers for WRC asthma. Data regarding the utility of the test in evaluating symptomatic workers is limited, but the reported high PPV for response to specific bronchial challenge in this population suggests some clinical potential for use in confirming the presence of WRC asthma in symptomatic workers. However, insufficient data exists to make firm recommendations about the usefulness of PA-HSA IgE RAST at the current time. Furthermore, in view of poor NPV, a negative test cannot be used to rule out WRC asthma in symptomatic workers.

CONCLUSION

For a medical test to be used effectively and appropriately, it is important to understand its performance characteristics. For example, a test with poor sensitivity is not ideal for use in medical screening, even if it has a high PPV. Performance characteristics of tests for antibodies specific to LMW agents in predicting asthma

caused by these agents differ greatly, depending on the specific LMW agent studied and the prevalence of asthma induced by that agent in the study population. In general, current published data supporting the use of tests to detect specific IgE and IgG to LMW agents in the diagnosis of occupational asthma caused by these agents is limited and inconclusive.

However, a few general statements can be made. The most promising data supporting use of a test for medical screening is for TMA-HSA IgE ELISA. Data assessing performance of tests measuring IgE and IgG to isocyanates and PA generally have shown poor sensitivity in predicting occupational asthma even in symptomatic exposed workers, so these tests appear unsuited for use in medical screening. Whether measuring specific antibodies to isocyanates and PA in symptomatic workers where occupational asthma is clinically suspected can be used to confirm disease remains unclear. A variety of factors related to the biology of asthma induced by isocyanates and PA and the technology of test performance might underlie poor sensitivity and inconsistent performance of these tests. In the case of isocyanates, determination of antigen-specific IgG might have some utility as a biomarker of exposure.

REFERENCES

1. Aul DJ, Bhaumik A, Kennedy AL, et al: Specific IgG response to monomeric and polymeric diphenylmethane diisocyanate conjugates in subjects with respiratory reactions to isocyanates. J Allergy Clin Immunol 103:749–755, 1999.
2. Baker DB, Gann PH, Brooks SM, et al: Cross-sectional study of platinum salts sensitization among precious metals refinery workers. Amer J Ind Med 18:653–664, 1990.
3. Banks DE, Butcher BT, Salvaggio JE: Isocyanate-induced respiratory disease. Ann Allergy 57:389–396, 1986.
4. Barker RD, Harris JM, Welch JA, et al: Occupational asthma caused by tetrachlorophthalic anhydride: A 12-year follow-up. J Allergy Clin Immunol 101:717–719, 1998.
5. Barker RD, van Tongeren MJ, Harris JM, et al: Risk factors for sensitization and respiratory symptoms among workers exposed to acid anhydrides: A cohort study. Occup Environ Med 55:684–691, 1998.
6. Bernstein JA: Overview of diisocyanate occupational asthma. Toxicol 111:181–189, 1996.
7. Bernstein JA, Munson J, Lummus ZL, et al: T-cell receptor Vβ gene segment expression in diisocyanate-induced occupational asthma. J Allergy Clin Immunol 99:245–250, 1997.
8. Brooks SM, Baker DB, Gann PH, et al: Cold air challenge and platinum skin reactivity in platinum refinery workers. Bronchial reactivity precedes skin prick response. Chest 97:1401–1407, 1990.
9. Calverley AE, Rees D, Dowdeswell RJ, et al: Platinum salt sensitivity in refinery workers: Incidence and effects of smoking and exposure. Occup Environ Med 52:661–666, 1995.
10. Cartier A, Grammer L, Malo JL, et al: Serum specific antibodies against isocyanates: Association with occupational asthma. J Allergy Clin Immunol 84:507–514, 1989.
11. Chan-Yeung M, Malo JL: Current concepts—Occupational asthma. New Engl J Med 333:107–112, 1995.
12. Chan-Yeung M: ACCP consensus statement. Assessment of asthma in the workplace. Chest 108:1084–1117, 1995.
13. Dykewicz MS, Patterson R, Harris KE: Induction of antigen-specific bronchial reactivity to trimellityl-human serum albumin by passive transfer of serum from humans to rhesus monkeys. J Lab Clin Med 111:459–465, 1988.
14. Frew A, Chan H, Dryden P, et al: Immunologic studies of the mechanisms of occupational asthma caused by western red cedar. J Allergy Clin Immunol 92:466–478, 1993.
15. Frew A, Chang JH, Chan H, et al: T-lymphocyte responses to plicatic acid-human serum albumin conjugate in occupational asthma caused by western red cedar. J Allergy Clin Immunol 101:841–847, 1998.
16. Grammer LC, Harris KE, Malo JL, et al: The use of an immunoassay index for antibodies against isocyanate human protein conjugates and application to human isocyanate disease. J Allergy Clin Immunol 86:94–98, 1990.
17. Grammer LC, Shaughnessy M, Kenamore B: Utility of antibody in identifying individuals who have or will develop anhydride-induced respiratory disease. Chest 114:1199–1202, 1998.
18. Howe W, Venables KM, Topping MD, et al. J Allergy Clin Immunol 71:5–11, 1983.

19. Karol MH, Tollerud DJ, Campbell TP, et al: Predictive value of airways hyperresponsiveness and circulating IgE for identifying types of responses to toluene diisocyanate inhalation challenge. Am J Respir Crit Care Med 149:611–615, 1994.
20. Lam S, Tan F, Chan H, Chan-Yeung MC: Relationship between types of asthmatic reaction, nonspecific bronchial reactivity, and specific IgE antibodies in patients with red cedar asthma. J Allergy Clin Immunol 72:134–139, 1983.
21. Liss GM, Bernstein D, Genesove L, et al: Assessment of risk factors for IgE-mediated sensitization to tetrachlorophthalic anhydride. J Allergy Clin Immunol 92:237–247, 1993.
22. Lowenthal M, Shaughnessy MA, Harris KE, Grammer LC: Immunologic cross-reactivity of acid-anhydrides with immunoglobulin E against trimellityl-human serum albumin. J Lab Clin Med 123:896–873, 1994.
23. Lushniak BD, Reh CM, Bernstein DI, Gallagher JS: Indirect assessment of 4,4'-diphenylmethane diisocyanate (MDI) exposure by evaluation of specific humoral responses to MDI conjugated to human serum albumin. Am J Ind Med 33:471–477, 1998.
24. Merget R, Schultze-Werninghaus G, Bode F, et al: Quantitative skin prick and bronchial provocation tests with platinum salt. Br J Indus Med 48:830–837, 1991.
25. Murdoch RD, Pepys J, Hughes EG: IgE antibody responses to platinum group metals: A large-scale refinery survey. Br J Ind Med 43:37–43, 1986.
26. Park HS, Kim HY, Nahm DH, et al: Specific IgG, but not specific IgE, antibodies to toluene diisocyanate-human serum albumin conjugate are associated with toluene diisocyanate bronchoprovocation test results. J Allergy Clin Immunol 104:847–851, 1999.
27. Postma DS, Bleecker ER: Genetics of asthma. In Barnes PJ, Grunstein MM, Leff AR, Woolcock AJ (eds): Asthma. Philadelphia, Lippincott-Raven, 1997, pp 145–154.
28. Raulf-Heimsoth M, Baur X: Pathomechanisms and pathophysiology of isocyanate-induced diseases—Summary of present knowledge. Amer J Ind Med 34:137–143, 1998.
29. Redlich CA, Homer RJ, Smith BR, et al: Immunologic responses to isocyanates in sensitized asthmatic subjects. Chest 109:6S–8S, 1996.
30. Redlich CA, Karol MH, Graham C, et al: Airway isocyanate-adducts in asthma induced by exposure to hexamethylene diisocyanate. Scan J Work Environ Health 23:227–231, 1997.
31. Sears MR: Risk factors. Immunoglobulin E and atopy. In Barnes PJ, Grunstein MM, Leff AR, Woolcock AJ (eds): Asthma. Philadelphia, Lippincott-Raven, 1997, pp 71–82.
32. Tamura G, Satoh K, Chao CL, et al: Do diagnostic procedures other than inhalation challenge predict immediate bronchial responses to inhaled allergen? Clin Exp Allergy 21:497–502, 1991.
33. Tarlo S: diisocyanate sensitization and antibody production. J Allergy Clin Immunol 103:739–741, 1999.
34. Tee RD, Cullinan P, Welch J, et al: Specific IgE to isocyanates: A useful diagnostic tool in occupational asthma. J Allergy Clin Immunol 101:709–715, 1998.
35. Tse KS, Chan H, Chan-Yeung M: Specific IgE antibodies in workers with occupational asthma due to western red cedar. Clin Allergy 12:249–258, 1982.
36. Vedal S, Chan-Yeung MC, Enarson DA, et al: Plicatic acid-specific IgE and nonspecific bronchial hyperresponsiveness in western red-cedar workers. J Allergy Clin Immunol 78:1103–1109, 1986.
37. Vedal S, Enarson DA, Chan H, et al: A longitudinal study of the occurrence of bronchial hyperresponsiveness in western red cedar workers. Am Rev Respir Dis 137:651–655, 1988.
38. Venables KM, Dally MB, Nunn AJ, et al: Smoking and occupational allergy in workers in a platinum refinery. Br Med J 299:939–946, 1989.
39. Venables KM, Chan-Yeung M: Occupational asthma. Lancet 349:1465–1469, 1997.
40. Welinder H, Nielsen J, Bensryd I, Skerfving S: IgG antibodies against polyisocyanates in car painters. Clin Allergy 18:85–93, 1988.
41. Young RP, Barker RD, Pile KD, et al: The association of HLA-DR3 with specific IgE to inhaled acid anhydrides. Am J Respir Crit Care Med 151:219–221, 1995.
42. Zeiss CR, Levitz D, Chacon R, et al: Quantitation and new antigenic determinant specificity of antibodies induced by inhalation of trimellitic anhydride in man. Int Archs Allergy Appl Immunol 61:380–388, 1980.
43. Zeiss CR, Wolkonsky P, Chacon R, et al: Syndromes in workers exposed to trimellitic anhydride. A longitudinal clinical and immunologic study. Ann Int Med 98:8–12, 1983.
44. Zeiss CR: Postgraduate course. Reactive chemicals in industrial asthma. J Allergy Clin Immunol 87:755–761, 1991.