Is Trimellitic Anhydride Skin Testing a Sufficient Screening Tool for Selectively Identifying TMA-Exposed Workers With TMA-Specific Serum IgE Antibodies?

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Objective: Trimellitic anhydride (TMA) can elicit specific IgE-mediated immune responses leading to asthma. This single-blinded study investigated the ability of TMA skin testing to identify workers with TMA-serum specific IgE antibodies. **Methods:** Forty TMA-exposed workers who were previously screened for the presence of TMA-IgG and/or IgE serum specific antibodies were skin tested to a TMA–human serum albumin reagent by nurses blinded to their antibody responses. **Results:** Findings from skin-prick tests were positive in 8 of 11 workers with TMA-serum specific IgE antibodies. Intracutaneous testing, performed only on skin prick testing–negative workers, was positive in two additional workers with TMA-serum specific IgE antibodies. A significant correlation was found between serum and skin test dilutions eliciting positive responses ($\rho = 0.87, P < 0.05; n = 11$). **Conclusions:** TMA skin testing provides an alternative and potentially more practical method for monitoring TMA-exposed workers for developing IgE sensitization.

Acid anhydrides are used for the production of plastics, paints, varnishes, and adhesives. Trimellitic anhydride (TMA) exposure may occur as dust and/or fume in the production phase where it is formed by catalytic oxidation of 1,2,4-trimethyl benzene or in packaging and logistic units. In TMA plants, personal exposure ranges from <0.6 to 1900 μg/mL.[1,2] It has been found that TMA becomes antigenic only after conjugation with larger endogenous carrier proteins such as human serum albumin (HSA), often by binding to lysine residues.[3,4] Inhaled TMA hapten, deposited on mucosal surfaces, reacts rapidly to form stable antigenic trimellityl-protein complexes.

The most common clinical effect of TMA is as an air toxic because it is rapidly converted to trimellitic acid, which causes respiratory irritation. Nevertheless, a unique characteristic of this low-molecular-weight chemical is that it is also capable of eliciting specific IgG- and IgE-mediated immune responses leading to asthma and other immune disorders.[5–8] The most common immunologic clinical conditions described for TMA-IgE sensitized workers have been rhinitis and asthma.[9] Once sensitized, symptoms can occur within minutes after TMA reexposure. Workers can also exhibit a latency period of weeks to years before the onset of symptoms.[3,5] Typically, reports of rhinitis and/or conjunctivitis precede the occurrence of asthma symptoms.[10] In a cross-sectional study conducted at a TMA-manufacturing plant with 474 employees, nearly 7% ($n = 32$) developed TMA-associated immune disorders consisting of asthma/rhinitis ($n = 12$), active late respiratory systemic syndrome ($n = 10$), late respiratory systemic syndrome in remission ($n = 5$), late onset asthma ($n = 4$), and late onset arthralgia ($n = 1$).[9]

Most immunosurveillance studies have used TMA-IgE and IgG serum specific antibody to predict the development of occupational respiratory disorders.[1,5,7,9,11] A limited number of studies have also used acid anhydride skin test reagents to relate worker sensitization to clinical disease and exposure.[12–14] Barker et al[12] demonstrated that a positive TMA skin test response was an independent risk factor for developing work-related bronchial hyperresponsiveness. In that study, other factors such as smoking history, atopic status (ie, sensitization to common inhalant allergens), and age were not significant after controlling for forced expiratory volume in 1 second.[12] Furthermore, although TMA skin testing was found to be associated with the development of serum-specific IgE antibody responses and subsequent clinical disease, no conclusive association was found with ambient TMA exposure concentrations, indicating that the magnitude of exposure was not the primary factor related to the development of sensitization.[12,15]

There are a few studies with limited numbers of subjects that investigated the association between acid anhydride skin testing and serum-specific IgE responses.[3,14,16] Two of these found a positive association between the acid anhydride skin test reagent and serum-specific IgE assays.[3,16] The study by Baur and Czuppon,[16] which evaluated workers exposed to a number of different acid anhydrides including TMA, found that the TMA-serum-specific IgE assay was more sensitive than skin prick testing (SPT).

Currently, industries that either manufacture TMA or use TMA in their work process have implemented rigid environmental control measures to reduce worker exposure. A major limitation of immunologic monitoring of workers for TMA sensitization is the cost associated with the need to regularly obtain serum for assessing increased TMA-specific IgE levels from potentially hundreds of workers. Furthermore, delays in obtaining the results of these serologic assays can potentially prolong clinical decisions regarding the worker's disposition in the workplace. Having a reliable TMA-specific skin test reagent could potentially enhance existing immunosurveillance programs as well as provide an alternative means for companies to cost-effectively monitor exposed workers for the development of TMA sensitization. Therefore, the purpose of this study was to determine whether the use of a well-characterized TMA-HSA skin test reagent could selectively identify workers with TMA-serum specific IgE antibodies.

METHODS

Subjects

This study describes data obtained from 40 workers who were recruited from a large TMA-manufacturing facility with an ongoing TMA-surveillance program. Before participation, workers signed an informed consent approved by the institutional review board at the University of Cincinnati College of Medicine. A modest compensation for participation was provided. Participants were selected on the basis of their TMA exposure, which was determined by their work

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process as either (1) no exposure (eg, administrative/office workers), (2) low exposure (eg, workers who rarely entered TMA areas), or (3) moderate or (4) high exposure, where the latter two categories included workers who were frequently or always exposed to TMA (eg, quality control engineers, packagers, and warehouse workers). All participants had been previously evaluated as part of an ongoing immunosurveillance program to determine whether they made TMA-specific IgG, IgG4, or IgE antibodies. Therefore, in selecting workers to participate in this study, it was possible to enroll a proportionate number of participants known to have no TMA-specific antibodies, TMA-serum specific IgG antibody only, or TMA-serum specific IgG and IgE antibodies. The nursing personnel performing the skin testing were blinded to the workers' TMA antibody and exposure status. Participants were tested over a 6-hour time period during a single day.

Quantitative Measurements for TMA-Specific IgG, IgG4, and IgE

The Phadia ImmunoCAP 1000 platform (Uppsala, Sweden) was used to measure TMA-specific IgG, IgG4, and IgE antibodies in TMA workers. The ImmunoCap System is based on the allergen of interest covalently coupled to a hydrophilic carrier polymer (cellulose) encased in a capsule. The allergen reacts with specific IgE in the sample and is detected with anti-IgE antibodies labeled with β-galactosidase. The fluorescent signal is then measured, with a higher signal indicating higher amounts of specific IgE in the sample.

Patient serum samples were analyzed following the manufacturer's instructions. For IgE testing, undiluted samples were used. For IgG and IgG4 testing, samples were diluted 1:100 using the manufacturer's sample diluent. Each immunoglobulin class has a six-point calibration curve. For the IgE assay, the calibrators are based on the WHO International Reference Preparation for human IgE 75/502 and are defined as international units (one international unit = 2.42 ng of IgE protein). The measured results in fluorescence units are evaluated against this calibration curve and expressed as concentration of allergen-specific units (kU/L). For the IgG assays, the calibrators are based on the WHO International Reference Preparation 67/86 for human serum immunoglobulins A, G, and M with results given in mass units. The limits of detection (LOD) for specific IgE for this assay are 0.10 to 100 kU/L, for specific IgG 2.0 to 200 μg/mL, and for specific IgG4 0.15 to 30 μg/mL.

Synthesis of TMA-HSA Conjugate for Skin Testing

The methods used to synthesize TMA-HSA skin test reagents were modifications of those previously described.[3,5] In brief, 100 mg of HSA was dissolved in 20 mL of 9% sodium bicarbonate and cooled to 4°C; 20 mg of TMA was then dissolved in 1 mL of acetone and rapidly added to a vigorously stirred HSA solution. The solution was stirred for 1 hour; unreacted TMA was removed by exhaustive dialysis, against changes of 4 L of 0.02 M ammonium bicarbonate at 4°C for 48 hours. All protein concentrations were determined by the bicinchoninic acid assay. The TMA-HSA conjugate was finally dialyzed against phosphate-buffered saline (pH 7.4) and reconstituted to a 2% (w/v) protein solution for skin testing.

The hapten-to-protein molar ratio (epitope density) was determined by MALDI-TOF (matrix-assisted laser desorption ionization–time of flight) mass spectrometry for better characterization of the conjugate using a Bruker Reflex IV MALDI-TOF instrument (Billerica, MA) and Sinapinic acid matrix. The incremental change in molecular weight due to conjugation of TMA to HSA was determined and the number of TMA molecules conjugated to HSA was calculated from the molecular weight of TMA.[17] Figure 1 illustrates the mass spectroscopy diagram of the TMA-HSA conjugate and the nonconjugated HSA carrier protein. It was found that the mass of the conjugate was 69343.9 Da compared with 65942.3 Da for HSA. The increase in mass due to conjugation was 3401.6 Da, resulting in a molar ratio of conjugation of 18.2.

Assessment of TMA-Specific IgE by Enzyme-Linked Immunosorbent Assay Using the TMA-HSA Conjugate

Serum obtained from a subset of workers with ($n = 6$) and without ($n = 7$) specific IgE reactivity to TMA-HSA previously identified by a commercial laboratory (ViraCor-IBT Laboratories, Lenexa, KS) was used to test the reactivity of the newly synthesized TMA-HSA conjugate by direct enzyme-linked immunosorbent assay (ELISA). The ELISA plates (Corning, New York) were coated with 100 μL of 100 μg/mL either TMA-HSA or HSA (control) in

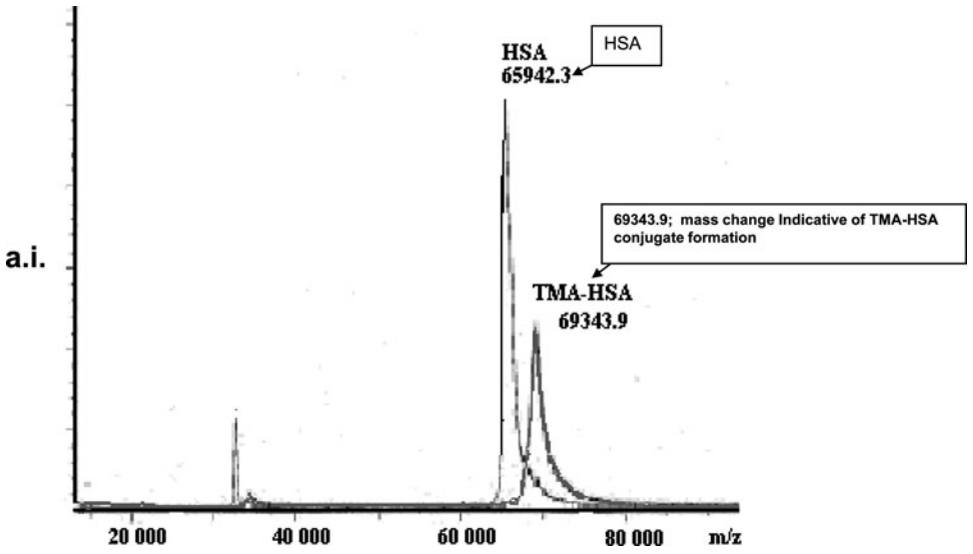

FIGURE 1. Analysis of conjugates by MALDI-TOF (matrix-assisted laser desorption ionization–time of flight) mass spectrometry. The shift in molecular weight (69343.9 − 65942.3 = 4301.6) indicates conjugate formation. The number of trimellitic anhydride (TMA) molecules (molecular weight = 192) bound to one human serum albumin (HAS) molecule (epitope density) was estimated to be 18.

carbonate buffer (pH 9.6) overnight at 4°C. The washing buffer between each step was tris-buffered saline solution with 0.05% Tween 20. One hundred microliters of the serially diluted (1/2, 1/4, 1/8, up to 1/1024) control and IgE-sensitized worker's serum were added and were incubated at 37°C for 60 minutes kept at 4°C for overnight. The following day, wells were washed and biotin-labeled antihuman IgE (1:1200; Southern Biotech, Birmingham, AL) was added and incubated for $2^{1}/_{2}$ hours at room temperature, followed by another $1^{1}/_{2}$-hour incubation with Sreptavidin-Alkaline phosphatase conjugate (1:1500; Southern Biotech). After washing, *p*-nitrophenyl phosphate (Sigma Chemical, St Louis, MO), 1 mg/mL in 10% diethanolamine buffer (pH 9.8) was added and incubated at 37°C until a positive control reached a predetermined optical density (OD). The OD at 405 nm was determined on a Bio-Tek automated model 312 Micro ELISA reader (Bio-Tek Instruments; Winooski, VT). A result was considered positive if the OD reading was 3 times or more the standard deviation of the negative controls. The research assistant performing these assays was blinded to which serum was positive or negative for TMA-specific IgE antibody.

Skin Testing of TMA-Unexposed Subjects to Identify an Irritant Response

Skin prick testing using dilutions of 1:100,000 (0.00001 mg/mL), 1:10,000 (0.0001 mg/mL), 1:1000 (0.001 mg/mL), 1:100 (0.01 mg/mL), 1:10 (0.1 mg/mL), 1:1 (1 mg/mL), and 5:1 (5 mg/mL) of the TMA-HSA conjugate was placed on 10 control participants without previous TMA exposure to identify irritant threshold responses in conjunction with a negative saline and positive histamine HCl (10 mg/mL) control. If SPT was negative, intracutaneous (IC) testing was performed beginning at a 1:100,000 w/v dilution of the 5 mg/mL solution (0.5×10^{-4}) down to 1:100 w/v (0.05 mg/mL) dilution to assess for an irritant response. A positive skin test was defined as a wheal of 3 mm or more, larger than the negative saline control in the presence of a positive histamine control.

Skin Testing TMA Workers

To determine whether skin testing using the well-characterized TMA skin test reagent could selectively identify TMA-serum specific IgE, SPT followed by IC testing only if the SPT was negative, was performed in conjunction with a negative saline and positive histamine (10 mg/mL) control on 40 workers with different TMA exposure levels and TMA-specific antibody profiles. The nursing personnel applying the skin tests were blinded to the TMA-specific antibody status of each worker. The wheal and flare responses of all positive skin test results were measured in millimeters and recorded using tracing tape; a wheal of 3 or more mm than a negative saline control was considered positive.

Statistical Methods

Fisher exact tests were analyzed to investigate associations of TMA exposure categories (moderate/high vs none/low), with (1) percentage of antibody responses greater than or equal to the LOD versus below the LOD for IgG, IgG4, and IgE and (2) SPT positivity. Sensitivity, specificity, and positive and negative predictive values were calculated to assess the ability of the combination of SPT^+ and SPT^- followed by IC^+ responses to predict specific IgE responses ($\geq$0.10 kU/L vs <0.10 kU/L). Log normal censored values of specific IgE and skin test dilutions were analyzed as well as log normally distributed (uncensored) values of total IgE. Correlation coefficients, adjusted for censoring, and 95% profile confidence limits were obtained to assess associations of continuously measured specific IgE with (1) total IgE and (2) skin test dilutions (if SPT^+ or IC^+ after SPT^-). The SAS procedures PROC FREQ and PROC NLP were used to obtain results (SAS for Windows, Version 9.2, Cary, NC).

RESULTS

Of the workers evaluated, the average age was $41^{1}/_{2}$ years and the majority were white men (36 men/4 women; 33 white/2 black/4 Hispanic/1 other). With respect to TMA exposure category levels, 3 had none, 10 had low, 16 had moderate, and 11 had high exposure (Table 1). Eleven workers had TMA-serum specific IgE, 10 of which also had TMA-serum specific IgG and/or IgG4 antibodies, whereas 17 workers had only TMA-serum specific IgG and/or IgG4 antibodies and 13 did not make TMA-serum specific antibodies.

Positive TMA-serum specific IgE antibody responses using the newly synthesized well-characterized TMA-HSA conjugate were observed using serum from workers with known TMA-serum specific IgE responses and were negative using serum from workers with a known negative TMA-serum specific IgE response (Figs. 2 and 3). It was determined that the ODs increased in linear proportion to a serum dilution range from 1:2 to 1:16, using serum from IgE-sensitized workers, not observed using serum from TMA-exposed, non–IgE-sensitized workers.

Skin prick testing followed by IC testing up to a 1:100 w/v dilution of 10 non–TMA-exposed healthy control participants was negative for all of the TMA-HSA concentrations applied.

Table 1 summarizes the results of TMA-HSA SPT^+ and SPT^-/IC^+ skin test responses, TMA-serum specific IgG, IgG4, and IgE responses and total IgE levels in workers with different TMA exposure levels. Not surprisingly, workers with higher TMA

TABLE 1. Percentage of Antibody Responses Above Limit of Detection for TMA-Specific IgG, IgG4, and IgE and Percentage of SPT Positive (SPT^+) and SPT^+ Combined With SPT^-/IC^+ Tests in 40 TMA Workers by Categories of TMA Exposure

TMA Exposure Level	Total Workers	Specific IgG*	Specific IgG4*	Specific IgE*	SPT^+†	SPT+ Combined With SPT^-/IC^+ Responders‡
N (%)	40 (100)	24 (60)	19 (47.5)	11 (27.5)	9 (22.5)	2 (5)
1 = none	3 (7.5)	0	0	0	0	0
2 = low	10 (25)	3 (7.5)	3 (7.5)	3 (7.5)	1 (2.5)	1 (2.5)
3 = moderate	16 (40)	13 (32.5)	10 (25)	1 (2.5)	2 (5)	0
4 = high	11 (27.5)	8 (20)	6 (15)	7 (17.5)	6 (15)	1 (2.5)

IC indicates intracutaneous; SPT, skin prick testing; TMA, trimellitic anhydride.
*Number (*N*[%] > LOD, the LOD of assay). For specific IgG, LOD = 2.0 μg/mL, specific IgG4, LOD = 0.15 μg/mL, specific IgE, LOD = 0.10 kU/L.
†Positive SPT (skin prick test) = positive wheal/flare reaction to a nonirritating concentration of TMA-HSA reagent $\leq$ 5 mg/mL (5:1 dilution).
‡IC testing was done only when SPT was negative. Positive IC (intracutaneous skin test) = positive wheal/flare reaction to a nonirritating concentration of TMA-HSA reagent $\leq$ 0.05 mg/mL (1:100 w/v dilution).

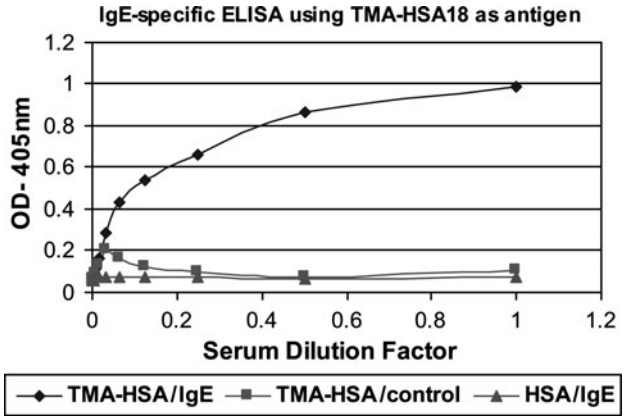

FIGURE 2. Enzyme-linked immunosorbent assay (ELISA) wells were coated with either trimellitic anhydride–human serum albumin (TMA-HSA) or HSA and incubated with a serially diluted using (1:2) control serum or serum from a worker with high TMA-specific IgE antibody. The optical density (OD) is plotted against the serum dilution factor. Only serum with high TMA-specific IgE antibody elicited a positive response to the TMA-HSA conjugate.

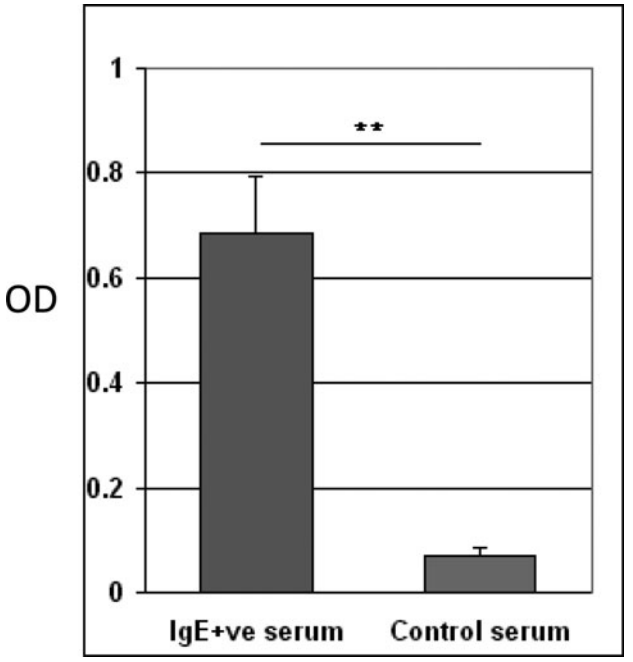

FIGURE 3. Results of IgE-specific direct enzyme-linked immunosorbent assay using trimellitic anhydride–human serum albumin (TMA-HSA) conjugate and serum samples collected from two groups of TMA-factory workers who were determined to be TMA (a) non–IgE-sensitized ($n = 7$) and (b) IgE-sensitized ($n = 6$). Specific IgE revealed approximately a 10-fold greater IgE optical density (OD) compared with the control group ($P < 0.01$).

exposure levels (levels 3 and 4) were more likely to produce TMA-specific IgG, IgG4, and IgE antibodies. However, workers with the highest TMA exposure levels (level 4) had the highest percentage of specific IgE serologic and skin test responses.

Three workers had positive TMA-serum specific IgE but a negative TMA SPT; however, two of these workers with a negative SPT response had a positive TMA IC tests at the 1:1000 w/v dilution. One worker with a negative TMA-serum specific IgE response had a positive TMA SPT. Of note, the two workers who exhibited a TMA-serum specific IgE response and a negative SPT followed by a positive IC response correlated well with moderate to high TMA exposure. Of the 11 workers with positive TMA-serum specific IgE or SPT responses, eight had a history of moderate to high TMA exposure (category 3 or 4) whereas three had a history of low exposure (category 2). No significant associations were detected between higher TMA exposure categories and percentage of antibody responses above the LODs for IgG, IgG4, IgE, or SPT positivity, although there was a positive trend for each analysis based on Fisher exact test.

Table 2 summarizes the sensitivity, specificity, and positive and negative predictive values of skin testing compared to serum-specific IgE testing. The serologic assay appeared to be more sensitive identifying 11 workers with specific IgE compared to SPT alone, which only identified nine workers. Two of these workers with TMA-serum specific IgE had a low-class 0/1 level, which may have been interpreted as a negative test. However, in an immunosurveillance program designed to detect early TMA sensitization, these equivocal tests should not be readily dismissed as they may represent the early signs of TMA sensitization. Using IC testing at a 1:1000 w/v dilution, if SPT was negative, increased the sensitivity of TMA-HSA skin testing from 73% to 91%. This indicates that when a worker has a positive skin test to the TMA-HSA reagent, he or she would also very likely have a positive serum-specific IgE test result. Of note, the specificity for TMA skin testing did not change with the addition of IC testing but the positive and negative predictive values did improve modestly. The high sensitivity and specificity of TMA SPT^+ combined with SPT^-/IC^+ responses indicate the very low likelihood of false-negative or false-positive test results, respectively

TABLE 2. Sensitivity, Specificity, Positive Predictive Values and Negative Predictive Values of (A) SPT (+, −) and (B) SPT Followed by IC Testing If Negative (+, −) for Predicting TMA-Specific IgE (+, −).

(A) SPT^+

	SPT*		
TMA-specific IgE†	+	−	All
≥0.10 kU/L	8	3	11
<0.10 kU/L	1	28	29
All	9	31	40

(B) SPT followed by IC testing if SPT is negative

	SPT^+	Combined With SPT^-/IC^+ Responses‡	
TMA-specific IgE	+	−	All
≥0.10 kU/L	10	1	11
<0.10 kU/L	1	28	29
All	11	29	40

IC indicates intracutaneous; SPT, skin prick testing; TMA, trimellitic anhydride.

*Positive SPT (skin prick test) = positive wheal/flare reaction to a nonirritating concentration of TMA-HSA reagent ≤ 5 mg/mL (5:1 w/v dilution). Sensitivity = 73%, specificity = 97%, PPV% = 89%, NPV% = 90%.

†TMA-specific IgE values were considered negative if < 0.10 kU/L and positive if ≥ 0.10 kU/L.

‡Positive IC (Intracutaneous skin test) = positive wheal/flare reaction to a nonirritating concentration of TMA-HSA reagent ≤ 0.01 mg/mL (1:100 w/v dilution). IC testing was administered only when SPT was negative. Sensitivity = 91%, specificity = 97%, PPV% = 91%, NPV% = 97%.

TABLE 3. Correlation Coefficients, Rho, (95% CL) for TMA-Serum Specific IgE and (1) Total IgE and (2) Minimum Skin Test Dilutions (SPT^+ Combined With SPT^-/IC^+ Responses)

Specific IgE (kU/L)	Rho (95% CL)	*P*
Total IgE* (IU/mL)	0.24 (− 0.15, 0.56)	>0.05
Minimum dilutions† (SPT^+ and SPT^-/IC^+ responses)	0.87 (0.79, 0.95)	<0.05

CL indicates confidence limits; IC, intracutaneous; IU, international unit; SPT, skin prick testing; TMA, trimellitic anhydride.
*$N = 11$.
†$N = 11$.

Finally, Table 3 summarizes the correlation coefficient for TMA-serum specific IgE and SPT^+ combined with SPT^-/IC^+ responses. Not surprisingly, the correlation coefficient (ρ) between these two tests was statistically significant at 0.87 ($P < 0.05$). Of note, there was no significant correlation between total IgE levels and TMA-serum specific IgE ($P > 0.05$).

CONCLUSIONS

The results of this study demonstrate that skin testing using a well-characterized TMA-HSA skin test reagent has excellent sensitivity and specificity as a test for selectively identifying TMA-sensitized workers compared to a very sensitive TMA-serum specific IgE immunoassay. Of note, these results confirm findings by other investigators that the TMA-serum specific IgE assays are more sensitive than TMA SPT.[16] Nevertheless, when a nonirritating IC skin test dilution is used when the SPT is negative, the sensitivity of this testing modality for identifying workers with TMA sensitization is significantly enhanced and comparable to an IgE-specific immunoassay.

This study is remarkable as it is the first definitive study to demonstrate that TMA skin test responses correlate well to TMA serum-specific IgE responses in a large number of TMA-exposed workers. Furthermore, the results of this study have important ramifications for companies that have TMA-exposed workers in the workplace but are unable to economically justify implementation of a comprehensive immunosurveillance program that would utilize TMA-serum specific immunoassays for monitoring exposure and sensitization.

A potential limitation of serologic assays that often occurs is how to interpret the clinical significance of low or equivocal specific IgE binding levels (ie, 0.1 to 0.35 kU/L). The serologic testing for TMA-specific IgE in this group of workers revealed that two workers were in this range that are frequently not considered clinically relevant because of previous studies that found poor correlation between low levels of sensitization and end organ provocation studies or manifestation of clinical disease.[18] Nevertheless, for the purpose of occupational immunosurveillance studies, it is very important to have very sensitive immunoassays as early detection of specific IgE sensitization is currently used as the paramount criteria for reducing or preventing further occupational exposure(s) to the worker. Interesting, one of these workers had a positive IC test at a nonirritating dilution of 1:1000 w/v or less confirming the reliability of the serologic test results that approached the LOD of the assay.

There are several advantages for incorporating skin testing into an immunosurveillance program. First and probably most important, skin testing provides an immediate result that permits more expeditious recommendations to be made regarding a worker's disposition. Second, as mentioned, TMA-SPT testing performed in conjunction with IC testing when SPT was negative increases the sensitivity for detecting TMA sensitization to 91% and the negative predictive value of testing to 97%, making it very unlikely to have a falsely negative test result that would miss the early onset of TMA sensitization in exposed workers. When skin testing (SPT^+ combined with SPT^-/IC^+ responses) and serologic testing are performed together, 100% of exposed workers with TMA-serum specific IgE sensitization are identified. Third, skin testing is easy and relatively cost-effective to administer. Finally, skin testing has the potential advantage of providing a better biologic equivalent of what is happening at the cellular level as it confirms that TMA-HSA specific IgE antibodies are binding to high-affinity IgE receptors on tissue mast cells.

In preparing a TMA-HSA skin test reagent, there are varying reports in the literature regarding the correct TMA:HSA molar ratio ranging from 18 to 20 to up to 300. Hapten-to-carrier ratio of the bioconjugate antigens has great influence on cellular response and development of immunoassays such as ELISA and radioallergosorbent test.[19] The rationale for a high molar ratio is to saturate the lysine residues on the protein carrier molecule (HSA or bovine serum albumin) to increase the sensitivity of the skin test reagent and/or immunoassay, but this can lead to false-positive results (reduced specificity).[3,16,20] Therefore, the rationale for a modest molar ratio is to prevent over-binding of TMA molecules on the carrier protein and reduce false-positive results. Similarly, some methods for preparing these conjugates initially dissolved TMA in acetone whereas others did not.[3,5,16,20] The methods used to synthesize the TMA-HSA conjugate in this study, modified from methods described by Zeiss et al and Bernstein et al, did not dissolve TMA in acetone and utilized a molar ratio of TMA:HSA of 18:1, which was able to be precisely confirmed using MALDI-TOF mass spectroscopy.[3,5] Utilization of proper controls (HSA and diluent) for the TMA-specific IgE ELISAs to confirm the specificity of the newly synthesized reagent demonstrated significant correlation with the immunoCAP commercial assay without evidence of nonspecific binding.

There are potential limitations of TMA skin testing in the workplace in that initial training is required to teach qualified health care personnel how to correctly prepare the skin test reagents for testing, perform SPT and IC testing as well as record and interpret skin test results. In addition, skin test reagents have to be kept frozen until used to maintain their stability over time. Finally, once a worker develops a positive skin test response, skin testing using a series of dilutions becomes necessary when monitoring for a decline in specific IgE antibody responses over time after the worker is removed from further exposure.

In summary, the results of this study demonstrate that using a TMA-HSA skin test reagent can be as sensitive and specific as a sensitive TMA-serum specific IgE immunoassay for detecting TMA-sensitized workers. Using both skin and serologic testing together would be ideal but is not always practical for employers who utilize TMA in the workplace. As the current standard of care is to remove sensitized workers from further TMA exposure to prevent development of rhinitis and asthma, TMA skin testing can provide an alternative and more practical method for longitudinally monitoring TMA-exposed workers for sensitization.

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