

Absence of relationship between degree of nonspecific and specific bronchial responsiveness in occupational asthma due to platinum salts

R. Merget, A. Dierkes, A. Rueckmann, E-M. Bergmann, G. Schultze-Werninghaus

Absence of relationship between degree of nonspecific and specific bronchial responsiveness in occupational asthma due to platinum salts. R. Merget, A. Dierkes, A. Rueckmann, E-M. Bergmann, G. Schultze-Werninghaus. ©ERS Journals Ltd 1996.

ABSTRACT: There is evidence that bronchial responsiveness to allergen is quantitatively correlated with bronchial responsiveness to nonspecific stimuli in subjects with allergic asthma. This association has been questioned in occupational asthma due to low molecular weight substances. It was the aim of this study to assess the quantitative association of bronchial responsiveness to methacholine (MCh) and platinum salts (Pt), in the form of hexachloroplatinic acid, in workers with occupational asthma due to platinum salts.

Fifty seven subjects with exposure to Pt, work-related asthma, and a positive bronchial challenge with Pt, underwent skin prick tests with Pt and bronchial challenge with MCh. Using the provocation concentration causing a $\geq 50\%$ fall in specific airway conductance ($PC_{50sGaw}(Pt)$) as dependent variable, anamnestic data (period from first symptoms to removal, period between removal from exposure and diagnosis, and smoking), season of the investigation, skin prick tests with environmental allergens, total immunoglobulin E (IgE), skin reactivity to Pt (Pt concentration causing a 2 mm wheal), and $PC_{50sGaw}(MCh)$ were included as independent variables for regression analysis.

Fifty two subjects (91%) showed a $PC_{50sGaw}(MCh) < 8 \text{ mg}\cdot\text{mL}^{-1}$ (geometric mean for all subjects $1.6 \text{ mg}\cdot\text{mL}^{-1}$). Responsiveness to Pt varied widely between subjects (geometric mean of $PC_{50sGaw} 9 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, range 2×10^{-7} to $10^{-2} \text{ mol}\cdot\text{L}^{-1}$). There was no univariate correlation between bronchial responsiveness to MCh and Pt, but there was a correlation between skin reactivity to Pt and $PC_{50sGaw}(Pt)$ ($r=0.6$). This association could not be improved by considering $PC_{50sGaw}(MCh)$, the period from first symptoms to removal, or the period between removal from exposure and diagnosis. The parameters that showed the highest (negative) associations with $PC_{50sGaw}(Pt)$ were skin reactivity to Pt and the period between removal from exposure and diagnosis ($r=0.65$).

We conclude that there is a moderate association between bronchial responsiveness to platinum salts and skin reactivity to platinum salts. However, there is no association between methacholine responsiveness and bronchial responsiveness to allergen in occupational asthma due to platinum salts.

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Division of Pneumology and Allergology, Dept of Internal Medicine, University Hospital 'Bergmannsheil', and Dept of Medical Computer Science, Biometrics and Epidemiology, Medical Division, Ruhr-University, Bochum, Germany.

Correspondence: R. Merget
Berufsgenossenschaftliche Kliniken
Bergmannsheil-Universitaetsklinik
Medizinische Klinik und Poliklinik
Abteilung Pneumologie und Allergologie
Buerkle-de-la-Camp-Platz 1
D-44789 Bochum
Germany

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Increased airway responsiveness to a variety of stimuli is part of the definition of asthma [1]. Sensitivity of testing with nonspecific stimuli is considered 100% in subjects with current asthma [2, 3], and only a few subjects considered to suffer from nonoccupational asthma without bronchial hyperresponsiveness have been described [4]. In contrast, the occurrence of variable airflow obstruction at a time of normal bronchial responsiveness to nonspecific stimuli was reported in a number of subjects with occupational asthma and exposure to low molecular weight substances [5–7]. Also, positive bronchial challenge tests with isocyanates have been described

in symptomatic subjects without increased responsiveness to methacholine [8]. The frequency of such cases has never been evaluated in epidemiological studies.

A quantitative association between airway responsiveness to nonspecific stimuli and allergens has been found for environmental allergens in most studies [9–12], although the association between skin test reactivity and bronchial responsiveness to allergen was closer [11]. Skin tests are not available for most low molecular weight substances, and studies concerning the association between bronchial responsiveness to the agent and nonspecific stimuli have given inconsistent results [13–15].

It is not known whether the lower association between specific and nonspecific bronchial hyperresponsiveness in occupational asthma is due to the nature of the substance (low or high molecular), the type of exposure (constant *vs* intermittent), or confounding factors, such as duration of symptomatic exposure or time without exposure prior to diagnosis.

In contrast to most low molecular weight substances, highly specific tests are available in platinum salt (Pt) asthma, and quantitative skin prick and bronchial provocation tests with the salt solutions can be performed, as with high molecular weight allergens, by inhalation of the substance with a nebulizer [16]. Platinum salts induce an immediate bronchial reaction in bronchial challenge tests, and dual or isolated late reactions rarely occur [16, 17]. The clinical picture of platinum salt asthma is identical with asthma due to environmental allergens.

It was the aim of this study to assess the association between bronchial responsiveness to methacholine (MCh), skin reactivity, and bronchial responsiveness to Pt, and to define the importance of possible confounding factors. It is important for clinical practice whether or not responsiveness to allergen can be predicted by nonspecific bronchial responsiveness, skin test reactivity, or a combination of both.

Methods

Subjects

A total of 57 platinum refinery and catalyst production workers were considered to have occupational asthma due to platinum salts (Pt). These workers were referred to our department for the evaluation of occupational asthma. All subjects gave a history of work-related asthma and showed an immediate bronchial reaction after challenge with Pt. No subject had asthma before being exposed to Pt. Forty subjects of this group (70%) reported asthma at the time of diagnosis. Thirty two subjects had been removed from exposure before diagnosis (mean 54 months, range 1–173 months). Exclusion criteria for the present study were: 1) medication; 2) severe airways obstruction; 3) technical reasons; 4) no stable phase; and 5) refusal. Thus, all subjects were without medication that might influence the test results, they were in a stable phase of their disease, and did not report acute viral infections during the last 2 weeks. All subjects agreed to participate in a longitudinal study that was approved by the Ethics Committee of the University [18].

History

Data concerning symptoms before exposure to Pt, work-related symptoms, present symptoms, smoking history period from removal to diagnosis, and time from the onset of symptoms to diagnosis were recorded. Asthma was defined as wheezing or shortness of breath on any occasion. It was not possible to quantify exposure. The workers reported variable symptoms at the workplace.

Skin prick tests

Skin prick tests were performed with a battery of environmental allergens (moulds, grass and tree pollen, animal dander, house dust mites; Allergopharma, Reinbek and Bencard, Neuss, Germany) according to the method of PEPYS [19]. Hexachloroplatinic acid (Sigma, Munich, Germany) 10^{-2} to 10^{-8} mol·L⁻¹ was used for skin prick tests with Pt as described previously [16]. As positivity criterion for skin tests we used a wheal diameter ≥ 4 mm with the commercial allergen extracts, or the 10^{-3} mol·L⁻¹ platinum salt solution. Skin reactivity to Pt was recorded as the Pt concentration that caused a 2 mm wheal diameter [12]. This was calculated from individual (log) concentration-response curves.

Total serum immunoglobulin E (IgE)

IgE levels were measured with Phadezym paper radioimmunosorbent test (PRIST)[®] (Pharmacia, Freiburg, Germany). Measurements were performed in duplicate, according to the manufacturer's directions.

Lung function and bronchial provocation tests

Forced expiratory volume in one second (FEV₁) was recorded with Masterlab (Jaeger, Wuerzburg, Germany). Measuring conditions were chosen as recommended by QUANJER *et al.* [20]. Bronchial hyperresponsiveness was assessed with MCh, as described previously [16], between 8 a.m. and 12 noon. MCh concentrations up to 50 mg·mL⁻¹ were used, thus it was possible to calculate the provocation concentration causing a $\geq 50\%$ fall in specific airway conductance (PC_{50sGaw}) in all tests. Bronchial hyperresponsiveness was defined as a PC_{50sGaw} of less than 8 mg·mL⁻¹ MCh.

Bronchial challenge tests with Pt were performed on the day after the methacholine challenge, as described previously [16]. Briefly, the same platinum salt solution as used in the skin test was inhaled with a jet nebulizer (Heyer, Bad Ems, Germany). Inhalation was performed with 10 breaths of 10 fold dilutions of platinum salt solutions at intervals of 15 min. Inhalations started at a concentration between 10^{-7} and 10^{-5} mol·L⁻¹. A positive test was defined as a PC_{50sGaw} with a Pt concentration $\leq 10^{-2}$ mol·L⁻¹ (highest concentration used). Peak flow measurements were recorded for 6 h with a mini-Wright peak flow meter (Airmed, London, UK), a late reaction was defined as a $\geq 20\%$ fall in peak flow with clinical symptoms after initial recovery from the immediate reaction.

Statistical analysis

Mean values are arithmetic means, with the exception of total IgE, the Pt concentration that caused a 2 mm wheal diameter in skin test, and PC_{50sGaw} for MCh and Pt (which are geometric means). For these parameters, logarithmic transformation was performed for calculations. In subjects without a wheal reaction in skin test

Table 1. — Personal data, skin prick tests with environmental allergens, total serum IgE and FEV₁

Subjects n	Sex M/F	Age yrs	Smoking		Period between			Subjects with environmental sensitizations %	IgE U·mL ⁻¹	FEV ₁ % pred
			pack-yrs	SS	first symptoms to diagnosis months	first symptoms to removal months	removal to diagnosis months			
Platinum salt asthma (all)	54/3	37±10 (18–59)	14±25 (0–180)	6 NS	63±64 (1–251)	33±52 (0–251)	30±47 (0–173)	49	102; 32/249 (7–1368)	98±14 (59–127)
				10 ExS 31 Smo				Perennial* Seasonal** 32		
Positive skin prick test with platinum salt	41/0	37±9 (23–59)	16±29 (0–180)	12 NS	70±72 (1–251)	40±59 (0–251)	30±52 (0–173)	44	112; 49/249 (7–1368)	99±14 (59–127)
				8 ExS 21 Smo				Perennial Seasonal 22		
Negative skin prick test with platinum salt	13/3	36±11 (18–57)	11±11 (0–35)	4 NS	44±30 (4–84)	12±12 (1–48)	30±31 (0–75)	63	80; 26/236 (9–881)	94±13 (74–110)
				3 ExS 10 Smo				Perennial Seasonal 56		

Values are presented as arithmetic mean±SD; with the exception of IgE (presented as geometric mean and 25/75% quartiles). Minimal and maximal values are given in parentheses. NS: nonsmoker; ExS: ex-smoker; M: male; F: female; IgE: immunoglobulin E; FEV₁: forced expiratory volume in one second; SS: smoking status. *: perennial allergens: house dust mite, animal dander, *Aspergillus spp.*, *Candida albicans*; **: seasonal allergens: grass and tree pollen, *Alternaria tenuis*.

(Pt), "10⁻¹" was inserted for the concentration of Pt causing a 2 mm wheal in order to avoid logarithmic transformation of zero values. Univariate associations were assessed by Pearson correlation coefficients. Differences between groups were compared using t-test or Fisher's exact test. All reported p-values are two-tailed. P values of 5% or less were considered significant. Least squares linear regression analysis was used to determine the relationship between parameters. The formula of COCKCROFT and co-workers [12] was adopted. The period from first symptoms to removal from exposure, and the period between removal from exposure and diagnosis were included in this model.

In a second step, stepwise regression with backward selection was performed by using PC50s_{Gaw}(Pt) as dependent variable, and the following parameters as independent variables: the period from first symptoms to removal from exposure; the period between removal from exposure and diagnosis; total serum IgE; skin reactivity to Pt; PC50s_{Gaw}(MCh); smoking status (smokers/nonsmokers; ex-smokers were summarized under smokers); skin prick tests with environmental allergens (positive/negative); sensitizations to seasonal allergens (positive/negative); and season of the investigation (February to August/September to January). Multiple linear regression analysis was performed with Statistical Analysis System (SAS) (SAS Institute Inc., Cary, NC USA), further calculations with Statgraphics (STSC Inc, Rockville, MA, USA).

Results

Skin tests with Pt showed a mean wheal diameter of 6.7 mm (range 0–16.5 mm) with the 10⁻³ mol·L⁻¹ solution. The mean Pt concentration causing a 2 mm wheal was 2×10⁻⁸ mol·L⁻¹ (range 5×10⁻⁹ to 3×10⁻³ mol·L⁻¹) for subjects with a wheal >0 mm. Forty one subjects showed a wheal ≥4 mm with the 10⁻³ mol·L⁻¹ solution. We identified 16 subjects who had a negative skin test with Pt, among whom were 11 subjects with no skin reaction at all. Anamnestic data (age, smoking status, period from removal to diagnosis, from first symptoms to removal, and from first symptoms to diagnosis) did not differ between skin test(Pt)-positives and skin test(Pt)-negatives. Furthermore, there were no differences in the number of positive skin tests with environmental allergens, IgE and FEV₁ (table 1).

No difference could be demonstrated in anamnestic data between subjects with symptoms at the time of diagnosis and those without, but the former had higher IgE (131, range 9–1368 vs 56, range 7–453 U·mL⁻¹, p<0.04), and lower FEV₁ at the time of diagnosis (95, range 59–127 vs 105, range 86–124% pred, p<0.02). Although total IgE was higher in symptomatic as compared to asymptomatic subjects the number of skin sensitizations to environmental allergens was lower (p<0.002). Total IgE was higher in subjects with environmental sensitizations as compared to subjects without further sensitizations (159, range 13–1,368 vs 66, range 7–1251 U·mL⁻¹, p<0.02).

There were no differences in any parameter between workers with ongoing exposure at the time of diagnosis and those who had been removed before diagnosis.

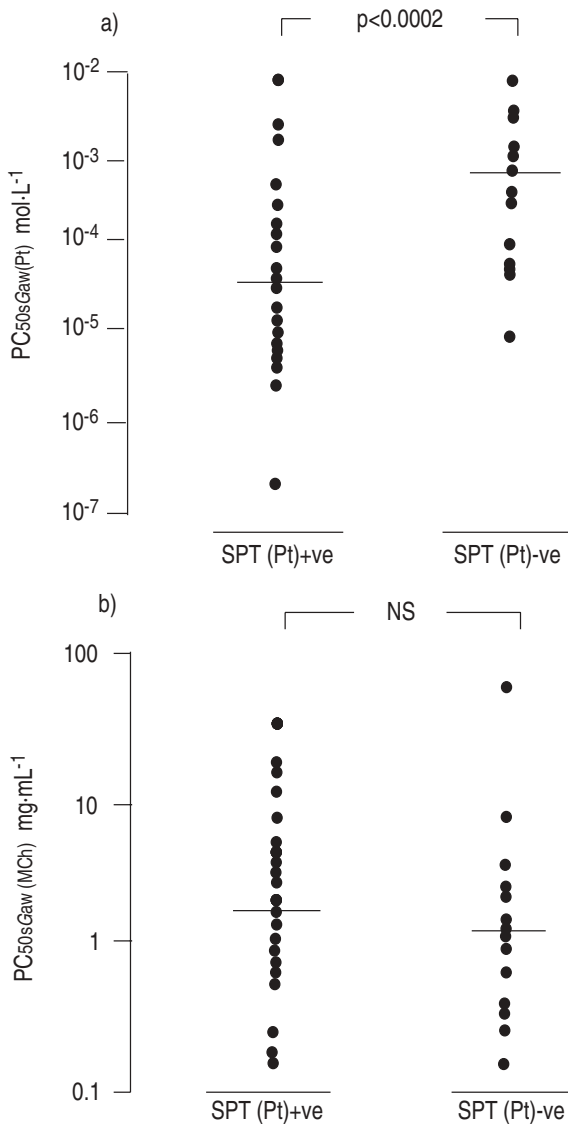


Fig. 1. – Bronchial responsiveness (PC_{50sGaw}) to Pt (a) and MCh (b) in workers with positive ($n=41$) and negative ($n=16$) skin prick tests (SPT) with Pt. PC_{50sGaw} : provocative concentration causing a $\geq 50\%$ fall in specific airway conductance; Pt: platinum salts; MCh: methacholine. The horizontal line represents the geometric mean.

Mean bronchial responsiveness to MCh was increased (PC_{50sGaw} 1.6, range 0.1–48 $mg \cdot mL^{-1}$). Only five subjects (9%) had no increased responsiveness to MCh, including four workers with present exposure. Bronchial responsiveness was increased in subjects with a positive skin test to environmental allergens as compared to non-atopics ($p < 0.03$). No difference could be demonstrated in $PC_{50sGaw}(MCh)$ between subjects with and without exposure at the time of diagnosis (2.1 vs 1.3 $mg \cdot mL^{-1}$; $p > 0.05$), subjects with or without symptoms at the time of diagnosis (1.5 vs 1.9 $mg \cdot mL^{-1}$; $p > 0.05$), and those with positive or negative skin test with Pt (1.8 vs 1.3 $mg \cdot mL^{-1}$; $p > 0.05$) (fig. 1). There was no association between skin reactivity to Pt and MCh responsiveness. Furthermore, no correlation was found between bronchial hyperresponsiveness or FEV₁ and the period from removal to diagnosis.

The concentration of Pt causing a 50% fall in specific

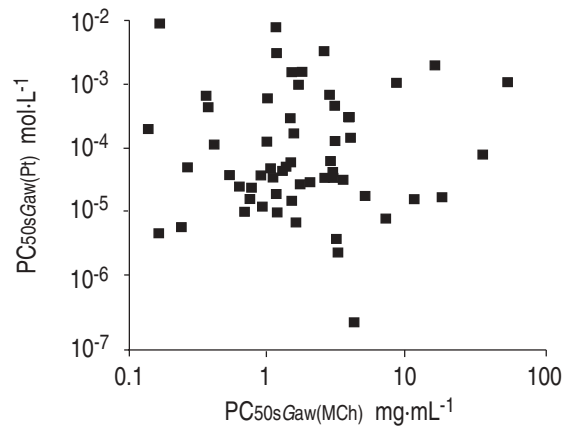


Fig. 2. – Correlation of bronchial responsiveness (PC_{50sGaw}) to Pt and MCh ($r=0.011$; $p=0.9$). For further abbreviations see legend to figure 1.

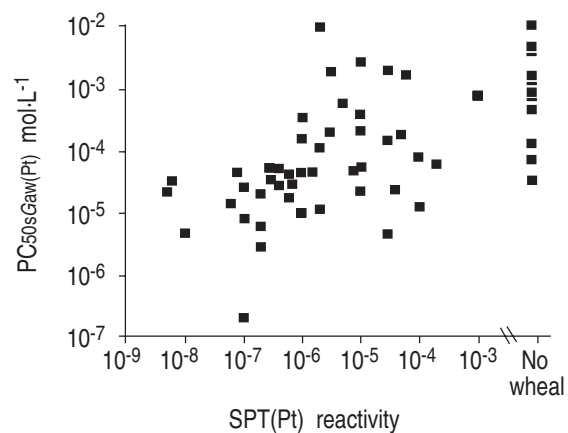


Fig. 3. – Correlation between bronchial responsiveness (PC_{50sGaw}) to Pt and skin reactivity to Pt (Pt concentration in $mol \cdot L^{-1}$ causing a 2mm wheal) ($r=0.6$; $p < 0.0001$). In skin test(Pt)-positives the correlation was smaller ($r=0.5$, $p < 0.002$). For further abbreviations see legend to figure 1.

airway conductance varied widely (range of $PC_{50sGaw}(Pt)$ 2×10^{-7} to $10^{-2} mol \cdot L^{-1}$). Skin test(Pt)-positives had a higher responsiveness to Pt as compared to skin test(Pt)-negatives ($p < 0.0002$), (fig. 1). Fifty one subjects showed an isolated immediate type reaction, and six subjects showed a dual asthmatic reaction. No difference could be demonstrated between subjects with and without exposure at the time of diagnosis (10^{-4} vs $7 \times 10^{-5} mol \cdot L^{-1}$; $p > 0.05$), or subjects with or without symptoms at the time of diagnosis (8×10^{-5} vs $10^{-4} mol \cdot L^{-1}$; $p > 0.05$).

Responsiveness to Pt was not correlated with MCh responsiveness ($r=0.011$; $p=0.9$), (fig. 2). There was a univariate correlation between skin reactivity and bronchial responsiveness to Pt ($r=0.6$; $p < 0.0001$), (fig. 3). If only subjects with a positive skin test were considered, the association was smaller but still significant ($r=0.5$; $p < 0.002$). There was a weak univariate negative correlation of $PC_{50sGaw}(Pt)$ with the period from removal to diagnosis ($r=-0.3$; $p < 0.02$).

Linear regression analysis confirmed a moderate association between $PC_{50sGaw}(Pt)$, $PC_{50sGaw}(MCh)$, and skin test reactivity to Pt ($r=0.6$), but the association was weak if only skin test(Pt)-positives were considered ($r=0.4$). The association could not be substantially increased by considering the period from first symptoms to removal and

Table 2. – Multiple linear regression analysis

Group	Model	Independent variables	Estimate	p-value	r
Whole group (n=57)	$\log_{10}PC50Pt=b_0+b_1\cdot\log_{10}(PC50MCh\cdot X)$	b_0	-2.818	0.0001	0.60
		b_1	0.265	0.0001	
Whole group (n=57)	$\log_{10}PC50Pt=b_0+b_1\cdot\log_{10}(PC50MCh\cdot X)+b_2\cdot Y+b_3\cdot Z$	b_0	-2.77	0.0001	0.66
		b_1	0.265	0.0001	
		b_2	-0.004	0.0459	
		b_3	0.003	0.1645	
Skin test (Pt)-positives (n=41)	$\log_{10}PC50Pt=b_0+b_1\cdot\log_{10}(PC50MCh\cdot X)$	b_0	-2.665	0.0001	0.40
		b_1	0.289	0.0092	
Skin test (Pt)-positives (n=41)	$\log_{10}PC50Pt=b_0+b_1\cdot\log_{10}(PC50MCh\cdot X)+b_2\cdot Y+b_3\cdot Z$	b_0	-3.119	0.0001	0.57
		b_1	0.207	0.0523	
		b_2	-0.005	0.0399	
		b_3	0.003	0.1001	

X: skin reactivity = Pt concentrations for a 2 mm wheal; Y: period from removal to diagnosis (months); Z: period from first symptoms to removal (months). For further abbreviations see legend to figure 1.

the period between removal from exposure and diagnosis ($r=0.66$ for the whole group; $r=0.57$ for skin test(Pt)-positives) (table 2). Performing stepwise regression with backward selection, the parameters that showed the highest (negative) associations with $PC50sGaw(Pt)$ were skin reactivity to Pt and the period between removal from exposure and diagnosis ($r=0.65$).

Discussion

It has been shown by immunohistological studies that T-lymphocyte activation and eosinophil recruitment are similar in occupational and nonoccupational asthma [21]. No qualitative differences between both types of asthma have been demonstrated by morphological or immunological methods. Bronchial hyperresponsiveness is closely associated with both types of asthma, although there is good evidence that the association of nonspecific and specific bronchial responsiveness is less close in occupational asthma due to low molecular weight substances [5–8, 13]. To our knowledge, this association has never been related to possible confounding factors, such as the pattern of exposure, the duration of the disease or to the period between removal from exposure and diagnosis. Most occupational allergens are low molecular weight substances, and it is not clear whether the putative difference of both types of asthma is due to the type of the substance, although similar airway inflammation has been found in occupational asthma after removal from exposure to low and high molecular weight substances [22].

In this study, we found no association of nonspecific and specific responsiveness in subjects with asthma due to a low molecular weight substance, which imitates asthma due to high molecular weight substances in many respects. We were unable to demonstrate a confounding effect of the duration of the disease, the period between removal from exposure and diagnosis, smoking, or skin prick test with environmental allergens on this association. This is an important finding, as removal from exposure of subjects with occupational asthma prior to the

tests could explain the weaker correlation between allergen and MCh responsiveness, if one assumes that the time course of recovery of responsiveness to allergen and nonspecific stimuli after removal differs.

$PC50sGaw(MCh)$ was lower in subjects with additional sensitizations to environmental allergens. These subjects also had a higher IgE as compared to subjects with sensitizations to Pt only. Symptomatic subjects had higher IgE, but fewer sensitizations to environmental allergens than asymptomatic subjects. Thus, environmental allergies were significant confounding factors for bronchial hyperresponsiveness, but not for symptoms of asthma (which occurred mostly at the workplace). There was no correlation between either lung function (FEV_1) or bronchial hyperresponsiveness and the duration of disease or the time without exposure. Surprisingly, the period from removal to diagnosis was negatively associated with $PC50sGaw(Pt)$, *i.e.* $PC50sGaw(Pt)$ increased with the period from removal to diagnosis. Whether this has to be interpreted as evidence of continuing negligible exposure in spite of removal cannot be concluded from these data. Removal from exposure was defined as being removed to areas within the plant, but outside the refinery. This did not exclude occasional contact to allergen *via* personal contact to refinery workers or contaminated materials. However, the association was small and, thus, might be by chance.

Taken together, we could not find an effect of confounding factors on the association between specific and nonspecific bronchial responsiveness, such as environmental allergies or the period without exposure before diagnosis; however, we cannot differentiate between the effect of the nature of the agent (low or high molecular) and the effect of the type of exposure (constant or intermittent).

A prediction of bronchial responsiveness to allergen from skin reactivity to allergen and airway responsiveness to histamine can be made for environmental high molecular weight substances [12]. We could not find an association between methacholine testing, skin reactivity to allergen, and bronchial challenge with allergen in platinum salt asthma. We are not aware of any study

using more indirect nonspecific stimuli, such as adenosine, as markers of nonspecific bronchial responsiveness.

The best predictor of airway responsiveness to allergen was skin reactivity to Pt, but this was mainly due to lower airway responsiveness to Pt in subjects with a negative skin test with Pt. A prediction of whether or not a subject will be found skin test(Pt)-positive by history is not possible. False positive bronchial challenge tests can be excluded, as controls were found to be not responsive to Pt with the identical method [16].

Another striking difference between platinum salt asthma and asthma due to environmental allergens was the wider range of PC50sGaw in this study as compared to published data in asthma due to environmental allergens. COCKCROFT and co-workers [12] reported a range of about 4,000 fold of provocation concentration causing a 20% fall in FEV₁ (PC20FEV₁) in bronchial challenge tests with environmental allergens. In Pt asthma, the range was about 100,000 fold. This difference cannot be explained by technical reasons, as the reproducibility of PC50sGaw(Pt) in longitudinal investigations was excellent [18]. The wide range of responsiveness to a low molecular weight substance is probably unique for platinum salts; in isocyanate asthma the range is about 20 fold [13, 14], and in subjects with red cedar asthma about five fold [15].

In summary, airway responsiveness to Pt and MCh were not found to be associated in this study. Furthermore, subjects without bronchial hyperresponsiveness to MCh, but with a clear asthmatic reaction after bronchial challenge with Pt were identified. We did not determine whether this difference is due to the nature of the allergen or the intermittent type of exposure in occupational asthma. This question should be addressed by studying occupational asthma due to high molecular weight substances. If bronchial challenge tests with platinum salts are performed, the starting concentration for bronchial challenge should be chosen independently from bronchial responsiveness to methacholine.

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