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The use of Induced Sputum as an Additional Diagnostic Indicator of an Asthmatic Reaction to Red Cedar after Specific Inhalation Challenge Testing

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WORK SAFE BC

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Original Project Title: Evaluation of electronically recorded peak flow monitoring and weekly change in spirometry as substitutes for inhalation challenge testing in red cedar asthma.

However, as explained in Research Problem subsection in the main body of the report, the project was revised to evaluate: The use of induced sputum as an additional diagnostic indicator of an asthmatic reaction to red cedar after specific inhalation challenge testing.

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1. MAIN RESEARCH FINDINGS:

- The aim of this study was to evaluate biomarkers of asthma activation in induced sputum before and after specific inhalation challenge testing with plicatic acid, to determine which biomarkers would be useful in confirming occupational asthma, when applied to workers both during work exposure and off work.
- In a total of 19 subjects with suspected red cedar asthma having red cedar inhalation challenge testing, sputum was induced with hypertonic saline about 6 hours after methacholine challenge on the control day and about 6 hours after plicatic acid inhalation challenge the next day.
- Eleven subjects developed bronchoconstriction after plicatic acid (Reactors), confirming the diagnosis of red cedar asthma, while 8 did not (Non-reactors), excluding red cedar asthma. In Reactors, after plicatic acid inhalation compared to control day, median values of sputum eosinophils increased from 6.5% to 25.0%, eosinophil cationic protein (ECP) increased from 260 to 456 ng/ml, and eotaxin from 58 to 133 pg/ml ($p < 0.01$), but LTB₄ and LTC₄ did not increase significantly. There were no significant changes in eosinophil counts. ECP, eotaxin, LTB₄, or LTC₄ in Non-reactors.
- **Conclusions:**
 - **1.** The markers of eosinophil activation, ECP and eotaxin, in induced sputum are better indicators of a positive reaction to specific inhalation challenge in red cedar asthma than leukotrienes.
 - **2.** The results suggest that sputum induction while subjects are working and after being off work for 2 weeks, to evaluate changes in eosinophil counts and %, as well as eotaxin, could confirm the diagnosis of red cedar asthma and reduce the requirement for red cedar inhalation challenge testing.

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2. EXECUTIVE SUMMARY

Western red cedar asthma is the most common occupational asthma in British Columbia, Canada. Diagnosis usually requires confirmation by the demonstration of immediate or delayed bronchoconstriction to inhalation of plicatic acid, the low molecular weight compound in red cedar wood implicated as the agent causing the asthma. The cedar inhalation challenge procedure requires 2½ days of testing, usually starting on Monday after the subject has been off work over the weekend. Subjects' asthma should be stable off inhaled bronchodilators, but inhaled corticosteroids are allowed to keep the asthma stable for testing.

On the first day of the cedar challenge testing, methacholine inhalation challenge is performed to determine non-specific bronchial reactivity, and spirometry and peak flow are followed in the Clinic Lab hourly for at least 6 hours after challenge. This serves a control day for the next day, when subjects are challenged with purified red cedar extract (plicatic acid). The subject monitors peak flow hourly at home until sleep time, and again the next morning on awakening and before leaving home; these again serve as control measurements for the 2nd day, the red cedar challenge day. On the 2nd day the subject is challenged with inhalation of plicatic acid and spirometry is followed every 10 minutes for the first hour to detect an immediate reaction, and then spirometry and peak flow are checked hourly for up to 6-7 hours to detect a sustained or delayed reaction. Again, subject monitors peak flow in the evening at home, and the next morning at home. Subject returns to the Clinic on the morning of the 3rd for recheck of spirometry and keep flow, and for reviewing test results.

The aim of this study was to evaluate biomarkers of asthma activation in induced sputum before and after specific inhalation challenge testing with plicatic acid, to determine which biomarkers would be useful in confirming occupational asthma, when applied to workers both during work exposure and off work.

During the study period, a total of 42 subjects were evaluated for suspected red cedar asthma by red cedar inhalation challenge testing. Out of these 42, 22 subjects consented to take part in the research study by having induced sputum on the control day and again on the red cedar extract (plicatic acid) challenge day, yielding a participation rate of 52 %. Sputum was induced with hypertonic saline about 6 hours after methacholine on the control day and about 6 hours after plicatic acid the next day. However induced sputum samples were not obtained on the plicatic acid challenge day in 3 subjects, leaving 19 subjects who had sputum induction before and after cedar challenge. Eleven subjects developed bronchoconstriction after plicatic acid (Reactors), confirming the diagnosis of red cedar asthma, while 8 did not (Non-reactors), excluding red cedar asthma. In one reactor, the sputum sample was frozen during transportation to the processing laboratory, leaving 18 subjects (10 Reactors and 8 Non-reactors) for comparison of sputum results before and after cedar challenge.

We determined cell counts and differential cell staining, and assayed eosinophil cationic protein (ECP), the leukotrienes LTB4 and LTC4, and eotaxin by specific ELISA. In Reactors, after plicatic acid inhalation compared with control day, median values of sputum eosinophils increased from 6.5% to 25.0% ($p < 0.001$), there was a trend for an increase in ECP from 260 to 456 ng/ml, and eotaxin increased from 58 to 133 pg/ml ($p < 0.001$). However, there was no consistent change in LTB4 and LTC4. Non-reactors had no consistent changes in any parameter.

We conclude that the markers of eosinophil activation, increased eosinophil counts and as % of cells, and increase in ECP and eotaxin, in induced sputum are better indicators of a positive reaction to specific inhalation challenge in red cedar asthma than leukotrienes.

The results suggest that sputum induction while subjects are working and after being off work for 2 weeks, to evaluate changes in eosinophil counts and %, as well as eotaxin, could confirm the diagnosis of red cedar asthma and reduce the requirement for red cedar inhalation challenge testing.

3. MAIN BODY OF REPORT

RESEARCH PROBLEM

The purpose of this research was to evaluate additional diagnostic procedures in evaluating red cedar asthma to reduce the necessity for performing red cedar inhalation challenges. Although the original title of this project indicates that the project was evaluating electronic peak flow monitoring and weekly monitoring of spirometry in occupational asthma, this evaluation was considered not feasible because there was a change in the referral pattern of subjects, with decrease in the number of referred subjects and most were outside the greater Vancouver area. The project was modified to evaluate the use of induced sputum (1,2), before and after specific inhalation challenge testing, as an additional diagnostic indicator of an asthmatic reaction to red cedar.

The project required a longer period of subject recruitment than the initially projected 2 years, for two reasons: 1) starting in 2003, there was a significant decline in the number of subjects referred for evaluation of red cedar asthma, 2) the project could not start until July 2005, after the UBC Ethics Board approved the use of induced sputum as a research procedure. The number of subjects referred for red cedar inhalation challenge testing remained low in 2005 and 2006, but the number increased significantly in 2007.

METHODOLOGY

Inhalation challenge testing for red cedar asthma. This follows the protocol standardized and established previously in our department by Dr. Chan-Yeung (3). In brief subjects were tested after being off work for at least 2 days, if their asthma was stable on treatment. They withheld inhaled bronchodilators, but were kept on their usual dose of inhaled corticosteroid. On the morning of the first day of testing they had methacholine inhalation testing to determine non-

specific bronchial reactivity. Their FEV₁ was then checked hourly with a computerized table top spirometer for at least 6 hr after completion of inhalation challenge; this served as a control day for the cedar challenge the next day. Peak flow was determined with a Mini-Wright peak flow meter hourly for the same time period, and then monitored hourly until sleep time, and then on waking up in the morning.

The next day, specific inhalation challenge testing was done using 2 minute nebulizations of plicatic acid (the compound in red cedar causing the asthma) starting at a concentration of 0.625 mg/ml with check of FEV₁ at 0.5 and 10 minutes. If there was no reaction, inhalation challenge and spirometry monitoring were repeated with nebulizations of doubling concentrations of plicatic acid up to the highest concentration of 10 mg/ml. The inhalation challenge was stopped if the subject developed a drop in FEV₁ of 20% or greater, or after the nebulization of the highest concentration of 10 mg/ml. FEV₁. Then FEV₁ and peak flow were monitored hourly for up to 6-7 hours after challenge. The patients then monitored their peak flow at home as on the previous day. The next morning, their peak flow recordings and symptoms were reviewed, and peak flow and FEV₁ were checked in the Clinic. They were then informed of the results of the testing and recommendations regarding work and treatment. A report was sent to the referring physician, to WorkSafeBC and to their family doctor.

Sputum induction as a research procedure. This was done after obtaining informed consent, and follows the technique developed in McMaster University (6) and utilized with slight modifications in our centre previously (1,5). The sputum induction was done 6 hrs after completion of methacholine nebulization, and up to 6-7 hrs after the plicatic acid challenge similar to previously (1), so as not to miss a late reaction. However, if the subject developed a

definite and sustained late reaction to plicatic acid, salbutamol inhalation would be given to control the broncho-constriction and then sputum induction would be started. Induced sputum was obtained after 7 minutes of nebulization of 3 % hypertonic saline, then 7 minutes of 4% saline, then if required after a 3rd nebulization of 5% saline. The subject's FEV₁ was monitored before and after each nebulization of hypertonic saline, and salbutamol by metered dose inhaler was given if the FEV₁ decreased by 10% or more. Sputum induction was stopped if the subject produced adequate amounts of mucus or was bothered by the procedure.

Processing and Analysis of induced sputum samples. The sputum samples were kept on ice packs and taken within 1½ hours to the Research Laboratory at St. Paul's Hospital as no trained technician was available at that time at VGH. The sample was processed throughout the study by the same technician trained at McMaster University in their protocol (6). The mucus was collected, weighed and suspended in dithiothreitol (DTT) solution to dissolve the mucus, and a cell count was determined. Cytospin smears were prepared for staining to determine differential cell counts. The mucus suspension was then centrifuged to obtain a cell free supernatant which was frozen in aliquots at -70 ° C for assay of asthma biomarkers. The remaining sample of induced sputum was frozen at -70 ° C for assay of biomarkers which might be affected by the DTT treatment.

Subjects tested. Inhalation challenge testing for red cedar asthma for all subjects was approved by WorkSafe BC, since the subjects' time off work and the cost of the inhalation challenge testing were covered by WCB. The research testing was approved by the University of B.C Ethics Review Board, and informed consent was obtained from all subjects. In 2005 and 2006 a total of 19 subjects had cedar inhalation challenge, of whom 9 consented to sputum induction for research purposes. In 2007 there was a significant increase in referrals, and a total of 23 subjects

had red cedar inhalation challenge. Of these, 13 consented to having induced sputum. The total number of subjects consenting to take part in the study was 22, out of a total of 42 subjects having cedar inhalation challenge, yielding a participation rate of 52 %. However, sputum before and after plicatic acid challenge was obtained in 19 subjects. Of these 11 reacted to plicatic acid confirming the diagnosis of red cedar asthma , while 8 did not, virtually excluding the diagnosis. However, in one subject (#5), the induced sputum sample on the control day was frozen during transportation to the processing Lab, rendering it unusable. This left only 18 samples (10 reactors and 8 non-reactors) suitable for comparison before and after challenge.

The number of subjects having induced sputum to evaluate red cedar asthma before and after challenge in this study, compares favourably with the two previous studies evaluating induced sputum in occupational asthma, one from Dr. Chan-Yeung's Lab in Vancouver (1) which evaluated 17 subjects with red cedar asthma, and the other by Lemiere et al.(2) at McMaster University, who evaluated 16 subjects.

Difficulties /challenges encountered. The major difficulty encountered previously was the low number of subjects referred for inhalation challenge for red cedar asthma during 2005 and 2006. However the number of referrals improved in 2007, and 20 patients were referred during this year, which is similar to our experience previously in 2002 and 2003. Another problem was the low rate of subjects accepting sputum induction among those undergoing inhalation challenge testing. This was partly due to a language barrier in some of the subjects making it difficult to use the informed consent form written in English. We tried to overcome this by the use of professional interpreters or family members to explain the procedures and the informed consent.

Analysis of Induced Sputum for markers of asthma activation. We used ELISA plates from MBL Int'l Corp for analysis of eosinophil cationic protein (ECP), from Cayman Chemical Inc. for analysis of LTB₄ & LTC₄ & cysteinyl leukotrienes (Cys-LT), and from R & D systems Inc. for eotaxin, following the manufacturers' procedure and standards, and as used in the literature (2, 7, 8). Samples and standards were done in duplicate; the assay was repeated with a different sample volume if the result did not fall in the recommended range of the standard curve. We were able to measure ECP, and LTB₄ and LTC₄ in the induced sputum samples treated with dithiothreitol (DTT), but were unable to detect eotaxin. This is because the DTT treatment resulted in degradation of eotaxin (9). However, we measured eotaxin in the remaining portions of the induced sputum samples not treated with DTT.

A problem with the technique of induced sputum preparation is that the DTT treatment may lead to disintegration of some biomarkers, and some mediators and cytokines may be completely lost after this treatment (9). Recently a study suggested treating the induced sputum supernatants with protease inhibitors to protect the biomarkers and removing the dithiothreitol by dialysis before any assays or storage at -70°C (10). However, this procedure is time consuming and not simple. A simpler procedure which we have now followed is to process two samples of sputum from the coughed out induced sample, one treated with DTT for the cell counts and differentials, and another without DTT for measuring biomarkers, as used by other investigators on expectorated sputum (11, 12).

Exhaled breath condensates (ECB). Collection of EBC has been suggested as a simple non-invasive technique for evaluating biomarkers of asthma activation in the exhaled breath (13). The technique is simple, where the subject breathes quietly for 10 to 20 minutes through a mouthpiece

and the exhaled breath passes into a cooling chamber, where the water vapor condenses, and the fluid can be collected for analysis of biomarkers. We have started collecting EBC for assaying bio-markers, such as eotaxin (14) and leukotrienes (15, 16) to indicate an asthmatic reaction. Reported levels in the literature in EBC for eotaxin (14) and for leukotrienes (15,16) in subjects with asthma were within the assay ranges of the ELISA kits we used. We used the system developed by Respiratory Research Inc. (www.respiratoryresearch.com ; Charlottesville, Va, USA) where the subject breathes quietly through a mouthpiece for at least 10 minutes, and the exhaled breath passes through a plastic collection tube, cooled by a metal jacket previously kept in a freezer, resulting in a collection of about 1.0 mL of fluid condensed from the breath over 10 minutes of quiet breathing. This is a very safe and well tolerated procedure without any risk. The advantage of this technique is that it can be used in the field and does not require a specialized laboratory for analysis within a few hours of sampling, as in the case of induced sputum, and the sample can be collected and kept frozen for later analysis in Vancouver.

Can Induced Sputum testing be used as an alternate to specific inhalation challenge ?

A multicentre study of occupational asthma from Eastern Canada (17) reported that induced sputum showed a reduction in eosinophils and eosinophil cationic protein in occupational asthma after a 2 week period off work compared to that obtained during work. They suggested that if the results of peak flow monitoring and induced sputum eosinophil counts at work and off work both support the diagnosis of occupational asthma, the results may be an alternate to specific inhalation challenge testing. In their study, they did not report the results of other markers of eosinophil activation. If further studies indicate that the use of induced sputum biomarkers of asthma activation as well as eosinophil counts, yields a high sensitivity and specificity in

diagnosing occupational asthma, it may become applicable as a tool for evaluating suspected occupational asthma at work and off work, without the need for inhalation challenge testing.

RESEARCH FINDINGS.

Table 1 compares mean data for subjects with confirmed red cedar asthma and that for subjects who did not react to plicatic acid and consequently did not have red cedar asthma. There was no significant difference in age, FEV₁, atopic status. Only one subject with red cedar asthma was a current smoker while 3 out of the 8 subjects with non-cedar asthma were current smokers.

The assays of induced sputum before and after plicatic acid challenge were completed in 18 subjects, because in one subject (#5), the induced sputum sample prior to plicatic acid challenge got frozen during transportation from our Clinic Lab to the Research Laboratory and was thus rendered unusable for diagnostic purposes, and so an induced sputum sample prior to plicatic acid challenge was not available for this subject. Instead, we obtained an induced sputum sample 28 hours after completion of the plicatic acid challenge when he had recovered from the asthmatic reaction; this was compared with the sample obtained 6 hours after challenge during the late asthmatic reaction, and the data for this subject are shown separately.

Fig 1 shows that there was a significant increase in neutrophil counts ($p < 0.01$) and eosinophil counts and eosinophil percentage ($p < 0.001$), all increased after plicatic acid challenge in patients with red cedar asthma, compared with induced sputum results on the control day prior to plicatic acid challenge, while there were no significant changes in the subjects who did not react to plicatic acid (data not shown). Fig 2 (top panel) shows that eosinophil cationic protein increased in all but 2 subjects having an asthmatic reaction to plicatic acid with an increase in the median values,

but the results were not statistically significant (Wilcoxon rank sign test). There was no consistent change in LTB₄. Median values for LTC₄ were higher after PA challenge, but the results were not statistically significant. There were no significant changes in the subjects who did not react to plicatic acid (Fig 2, bottom panel).

Eotaxin could not be detected in the induced sputum samples treated with DTT. However we measured eotaxin levels in the remaining part of the induced sputum sample not treated with DTT, and simultaneously measured LTB₄ to calculate the eotaxin/LTB₄ ratio. Fig 3 shows that in the subjects with red cedar asthma there was a significant increase ($p < 0.001$) in eotaxin levels in induced sputum after plicatic acid challenge, while there were no significant changes in LTB₄. There was also a significant increase in the eotaxin/LTB₄ ratio ($p < 0.001$) after plicatic acid challenge

The subject (# 5) whose baseline pre-challenge induced sputum was not usable for analysis because it got frozen during transportation, reacted to plicatic acid challenge confirming the diagnosis of red cedar asthma. He had high levels of neutrophil and eosinophil counts in induced sputum as well as elevated ECP, LTB₄, LTC₄, and eotaxin levels 6 hours after challenge (Table 2). All values except the ECP had decreased the next day when he was recovering from his asthmatic reaction 28 hours after inhalation (Table 2). It is likely that ECP remained high, presumably because he still had elevated eosinophils, which were releasing ECP. It is very likely that his levels would have been even lower in the sputum obtained on the control day had it not been rendered unusable by freezing.

Our results indicate that evaluation of induced sputum provides confirmation of a positive bronchial reaction to specific inhalation challenge testing in the diagnosis of occupational asthma. Induced sputum results could provide additional evidence to support a positive reaction to inhalation challenge testing in subjects who develop a borderline bronchial reaction (FEV₁ response) to plicatic acid. The cell counts and differential cell counts showed a significant increase in eosinophil counts in Reactors as compared to Non-reactors to red cedar asthma extract. The majority of Reactors showed an increase in eosinophilic cationic protein (ECP), a biomarker released from eosinophils. All reactors showed an increase in eotaxin, a chemo-attractant for eosinophils released during the asthmatic reaction. There were no significant increases in LTB₄ or LTC₄ following the late reaction to plicatic acid. The response in red cedar asthma differs from that reported in isocyanate-induced asthma where there was release of LTB₄ (7); however isocyanate asthma may initiate a predominantly neutrophilic rather than eosinophilic cellular response.

Results of assays of asthma biomarkers in Exhaled Breath Condensates (EBC)

We were unable to detect eotaxin or leukotrienes in EBC of asthma subjects having a positive reaction to plicatic acid. However, eotaxin and leukotrienes in EBC were readily detected in European centres (14-16), where EBC was collected by specialised apparatus (e.g. Jaeger Eco-Screen), where the exhaled breath condenses in a flask kept cooled by a refrigerating unit. Failla et al (16) reviewed the literature and reported median values for cysteinyl-leukotrienes (cys-LT) of about 20 pg/ml in normal subjects, increasing to about 60 in allergic rhinitis, and higher to about 70 in asthma. In contrast, Debley and co-workers (18), using the RT-Tube of Respiratory Research (the system we tried) reported that EBC had to be concentrated 6 to 12 fold to enable

measurement of cys-LT; they reported median values in adults with mild asthma of 4.3 pg/ml and of 1.5 pg/ml in healthy adults.

A possibility we considered was that there could be loss of biomarkers due to their adhesion to the plastic collection tubes. We checked this, by comparing levels of biomarkers collected from a few normal volunteers and asthma subjects, in tubes with and without prior coating with 1% bovine serum albumin and 0.01% Tween 20, as recommended by two investigators from Sweden (19). Preliminary results indicate that eotaxin was detectable at very low levels in unconcentrated EBC collected in coated tubes but not in uncoated tubes from asthma patients, indicating that coating prevents some loss of biomarkers. However, there may be other factors affecting results collected by the RT tubes. Another possibility to be considered is that the cooling effect of the metal jacket does not last long enough and is not sufficient to prevent degradation of biomarkers during the 10 or 20 minutes of collection. More evaluation and quality control procedures are needed to standardize the methodology of collecting EBC and its reproducibility, before it can be applied for evaluation of occupational asthma. However, since this is a non-invasive technique to obtain biomarkers from the respiratory tract, and since European reports are encouraging, further research is warranted.

Conclusions.

Induced sputum as a research procedure was obtained before and after plicatic acid challenge in 18 subjects undergoing inhalation challenge testing for suspected red cedar asthma. Ten subjects had reacted to the inhalation challenge testing with a significant decrease in FEV₁ confirming the diagnosis of red cedar asthma, while 8 did not, virtually excluding the diagnosis. The reactors showed an increase in neutrophil counts and highly significant increases in eosinophil counts and

% eosinophils in induced sputum after plicatic acid challenge. There was a trend for an increase in eosinophilic cationic protein (ECP), and a highly significant increase in eotaxin, but no significant increase in the leukotrienes LTB₄ and LTC₄. These results indicate that markers of eosinophilic activation in induced sputum are better indicators of a positive asthmatic reaction to plicatic acid than the leukotrienes.

IMPLICATIONS for FUTURE RESEARCH ON OCCUPATIONAL ASTHMA

Our results suggest that using the combination of changes in eosinophil counts and percentage of cells, and eotaxin concentration, and possibly ECP before and after challenge would be good indicators of predicting an asthmatic reaction to plicatic acid. We will obtain statistical consultation to evaluate the sensitivity and specificity of these biomarkers separately and in combination, in diagnosing red cedar asthma.

Establishing induced sputum at work and off work as an alternate to specific inhalation challenge testing may require a trial comparing induced sputum results thus obtained, with subsequent cedar inhalation challenge testing.

POLICY and PREVENTION

It appears from some workers' descriptions that some mills have high dust levels and poor ventilation and may not be meeting WorkSafeBC standards. This project was not specifically designed to assess policy or prevention.

DISSEMINATION/KNOWLEDGE TRANSFER

R.A. presented this research project in detail at the UBC Wednesday (VGH-St Paul's) Respiratory Medicine Seminars on 19 March 2008, and it was well received and generated interest in the use of induced sputum biomarkers as an alternate to specific inhalation challenge testing. A poster is being presented at the "Lung Health Research and Policy Day" Meeting to be held at the Sheraton Wall Center Hotel on 27 March 2008 (Abstract attached at end of this report). An Abstract has been submitted to the European Respiratory Society for consideration for presentation at their meeting in October 2008 in Berlin (Abstract attached at end). R.A. is also planning to submit an Abstract for presentation to the Canadian Respiratory Clinical Research Consortium Meeting held on 19 June 2008 in Montreal in conjunction with the 1st Canadian Respiratory Conference 19-21 June 2008.

A paper will be prepared for submission to one of the prime journals in Respiratory Medicine. It is planned to present the results of this study at a Canadian Lung Conference this summer and to the European Respiratory Society in Berlin in October 2008. Copies of the published article will be submitted to WorkSafe BC.

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Table 1: Comparison of subjects with confirmed red cedar asthma reacting to plicatic acid challenge, and those with asthma not due to red cedar (negative challenge)

	Positive Challenge	Negative Challenge
No of Subjects	11	8
Age Mean (SD)	37.9 (8.1)	41.6 (12.9)
Male (%)	100	87.5
Smoking Status		
Never Smoked (%)	80.0	62.5
Current Smoker (%)	10.0	37.5
Atopy: Positive Skin Test %	40	50
Yrs of Exposure to Cedar Mean (SD)	5.4 (4.2)	5.4(3.7)
<u>Baseline Lung Function</u>		
FEV1 L Mean (SD)	3.6 (0.7)	3.47 (1.15)
FEV ₁ % Predicted Mean (SD)	84.9 (13.3)	100.5 (18.4)
<u>Methacholine Challenge</u>		
% positive	90	50
<u>Plicatic acid Challenge</u>		
Positive Immediate Reaction %	50	0
Positive Late Reaction (%)	100	0

Table 2: Induced sputum results in Subject # 5 with red cedar asthma, 6 hrs and 28 hrs after plicatic acid inhalation challenge.

	Neutrophil count 1000/mg sputum	Eosinophil count 1000/mg sputum	% Eosino- phils	ECP ng/mL	LTB4 pg/mL	LTC4 pg/mL	Eotaxin pg/mL
6 hrs after plicatic acid challenge	15.2	7.49	26.8	642	1198	504	104
28 hrs after plicatic acid challenge	2.9	1.51	17.2	649	236	106	64

LEGENDS

Figure 1. Neutrophil count and eosinophil count in induced sputum in the patients with red cedar asthma before and after plicatic acid challenge. (Bars indicate median values)

There was a significant increase in neutrophil ($p < 0.01$) and eosinophil counts ($p < 0.001$), and in the percentage of eosinophils ($p < 0.001$) after plicatic acid challenge in the subjects reacting to plicatic acid. In contrast, there were no significant increases in neutrophil or eosinophil counts in the subjects who did not react to plicatic acid (data not shown).

Figure 2. Eosinophil cationic protein (ECP), LTB₄, and LTC₄ in induced sputum in subjects with and without red cedar asthma, before and after inhalation challenge with plicatic acid. (Bars indicate median values)

Top panel: subjects with confirmed red cedar asthma: There was a trend for an increase in ECP after inhalation challenge with plicatic acid, no change in LTB₄, and a non-significant changes in LTC₄.

Bottom panel: subjects with asthma not due to red cedar: There was no consistent change in any of the mediators

Figure 3 LTB₄ and Eotaxin levels in induced sputum not treated with dithiothreitol, in subjects with red cedar asthma before and after plicatic acid challenge:

There was no significant change in LTB₄, but there was a statistically significant increase in eotaxin levels ($p < 0.001$) and in the eotaxin/LTB₄ ratio ($p < 0.001$), after plicatic acid challenge in subjects with red cedar asthma.

Figure 1

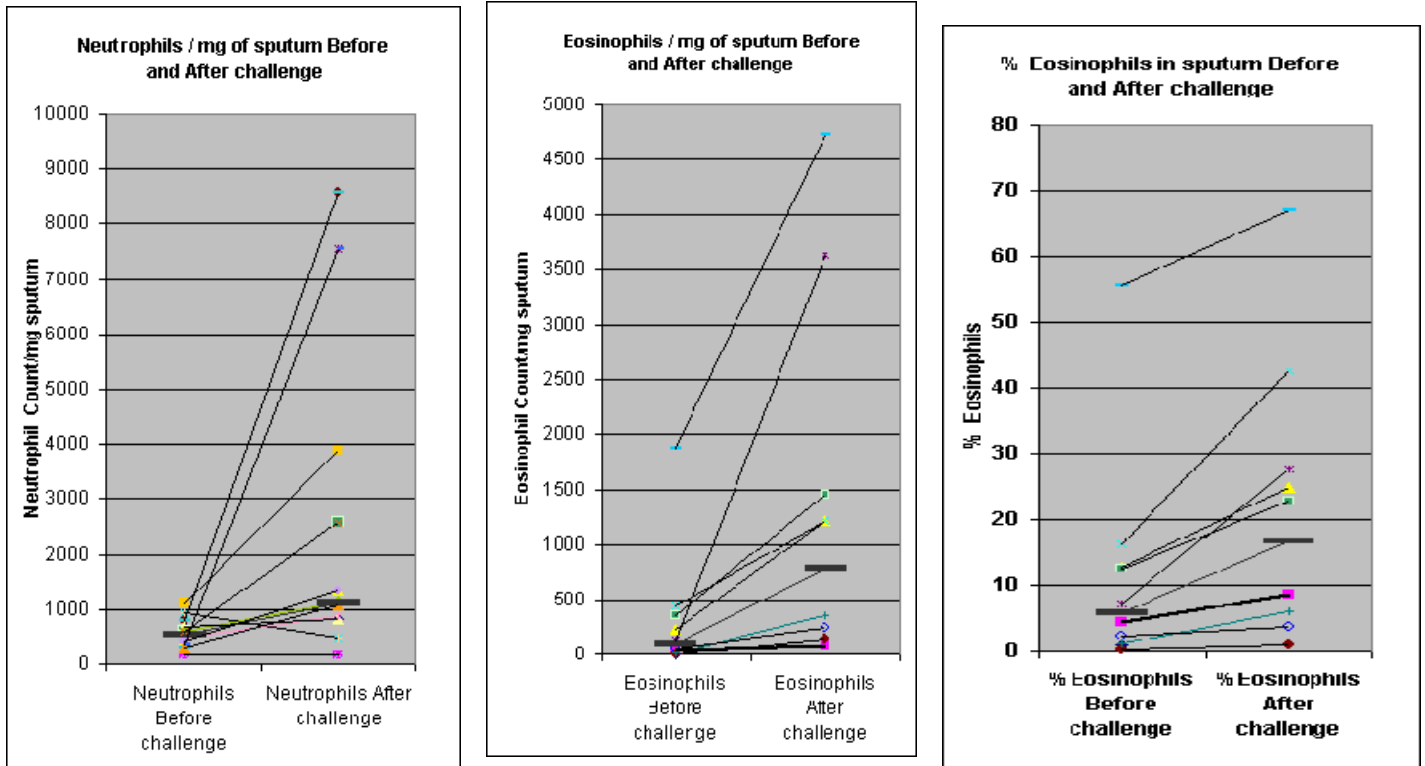
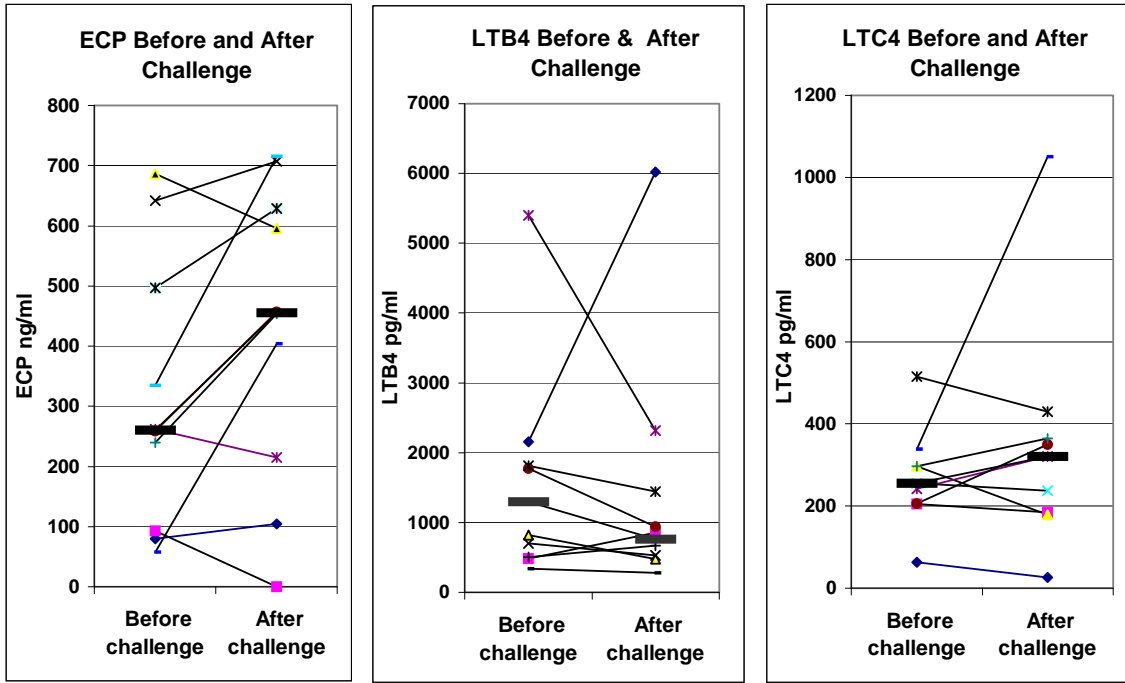
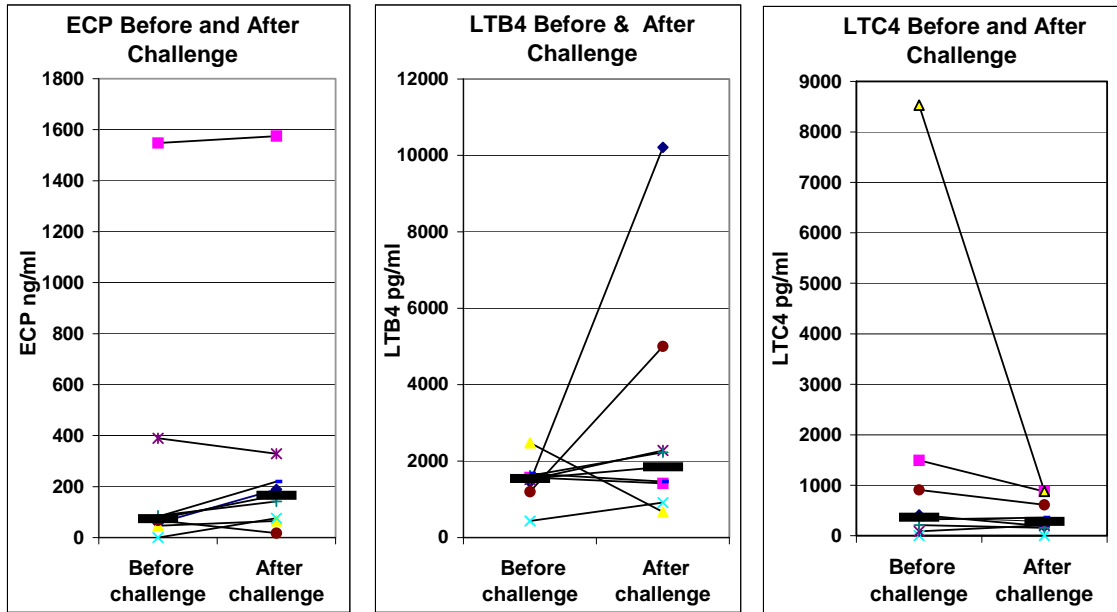


Figure-2

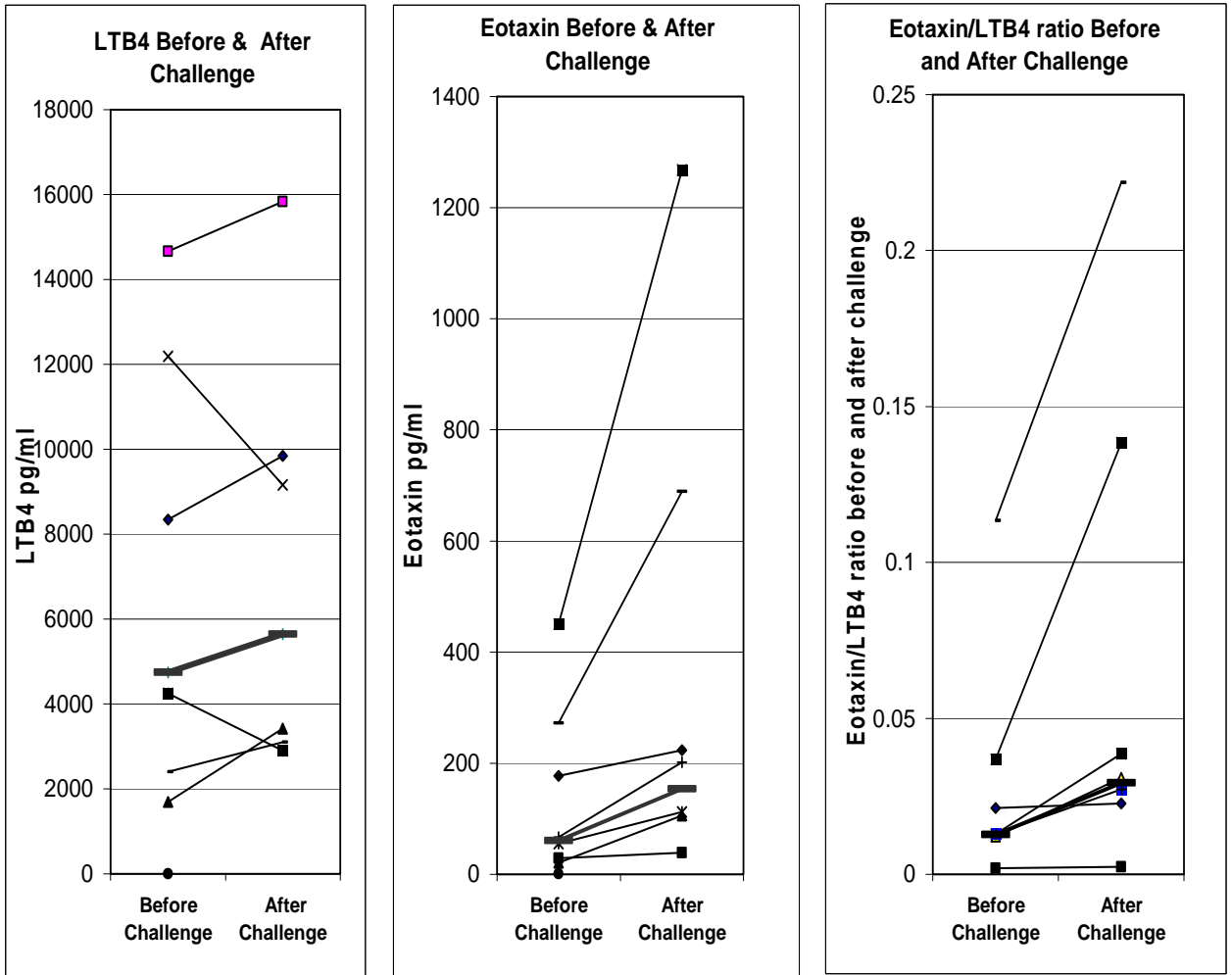


ECP, LTB4 and LTC4 in reactors



ECP, LTB4 and LTC4 in non reactors

Figure 3. Eotaxin and LTB4 levels in induced sputum not treated with dithiothreitol.



Abstract for Poster Presentation at

Centre for Lung Health Research & Policy Day, 27 Mar 08, Sheraton Wall Centre

Title: Eosinophil cationic protein and eotaxin are better biomarkers of asthma activation than leukotrienes in induced sputum after specific inhalation challenge testing in subjects with western red cedar asthma

Authors: Raja Abboud MD FRCPC, Selvarani Vimalanathan, PhD, Moira Chan-Yeung MD FRCPC, Respiratory Medicine Division, University of British Columbia at Vancouver General Hospital, and Vancouver Coastal Health Research Institute, Vancouver, B.C.

Abstract: Western red cedar asthma is the most common occupational asthma in British Columbia. The diagnosis is usually confirmed by the demonstration of immediate or delayed bronchoconstriction to inhalation of plicatic acid, the low molecular weight compound causing the asthma (Chan-Yeung M, *et al.* Am J Med 1982; 72: 25-29). Our aim was to evaluate biomarkers of asthma activation in induced sputum before and after specific inhalation challenge testing with plicatic acid, to determine which biomarkers would be useful in confirming occupational red cedar asthma. Testing for these biomarkers in induced sputum in workers exposed to red cedar both during work exposure and off work, could then be used to confirm the diagnosis without performing the time consuming inhalation challenge test with plicatic acid and risking side effects.

We studied a total of 18 subjects with suspected red cedar asthma who had specific inhalation challenge testing. Induced sputum was obtained after informed consent, by ultrasonic nebulization of hypertonic saline, 6 hours after methacholine on the control day, and 6 hours after plicatic acid the next day. Ten subjects developed bronchoconstriction after plicatic acid (Reactors), confirming the diagnosis of red cedar asthma, while 8 did not (Non-reactors) excluding the diagnosis. In Reactors, median values in induced sputum after plicatic acid inhalation compared to the control day increased as follows: sputum eosinophils from 6.5% to 25.0%, eosinophil cationic protein (ECP) from 260 to 456 ng/ml, and eotaxin from 58 to 133 pg/ml ($p < 0.01$). However, there were no significant increases in the leukotrienes LTB₄ and LTC₄. Non-reactors did not show any significant changes in any parameter. We conclude that the markers of eosinophil activation, ECP and eotaxin, are better biomarkers than leukotrienes of a positive reaction to specific inhalation challenge in red cedar asthma.

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Abstract Submitted to European Respiratory Society Meeting Oct 2008

Title: Evaluation of biomarkers of asthma activation in induced sputum before and after specific inhalation challenge testing for western red cedar asthma

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Body: Western red cedar asthma is the most common occupational asthma in British Columbia, Canada. Diagnosis often requires confirmation by the demonstration of immediate or delayed bronchoconstriction to inhalation of plicatic acid, the low molecular weight compound implicated as the causative agent (Chan-Yeung M, *et al.* Am J Med 1982; 72: 25-29). The aim of this study was to evaluate biomarkers of asthma activation in induced sputum before and after specific inhalation challenge testing with plicatic acid, to determine which biomarkers would be useful in confirming occupational asthma, when applied to workers both during work exposure and off work.

Induced sputum was obtained after informed consent from a total of 18 subjects with suspected red cedar asthma having inhalation challenge testing. Sputum was induced with hypertonic saline 6 hours both after methacholine on the control day and plicatic acid the next day. Ten subjects developed bronchoconstriction after plicatic acid (Reactors), confirming the diagnosis of red cedar asthma, while 8 did not (Non-reactors). In Reactors, after plicatic acid inhalation compared to control day, median values of sputum eosinophils increased from 6.5% to 25.0%, eosinophil cationic protein (ECP) increased from 260 to 456 ng/ml, and eotaxin from 58 to 133 pg/ml ($p < 0.01$), but LTB₄ and LTC₄ did not increase significantly. Non-reactors did not show any significant changes in any parameter. We conclude that the markers of eosinophil activation, ECP and eotaxin, are better indicators of a positive reaction to specific inhalation challenge in red cedar asthma than leukotrienes.

Supported by a WorkSafeBC grant