Normal Range of Methacholine Responsiveness in Relation to Prechallenge Pulmonary Function*

The Normative Aging Study

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Methacholine airway responsiveness has been observed to be related to prechallenge level of pulmonary function; however, normal ranges of responsiveness for specific levels of lung function have not been reported. We examined methacholine airway responsiveness in relation to level of prechallenge pulmonary function in a sample of 547 middle-aged and elderly men who denied any history of respiratory illness or symptoms and who had normal levels of prechallenge FEV\textsubscript{1}, and FEV\textsubscript{1}/FVC ratio. The cumulative dose of methacholine provoking a 20 percent decline in FEV\textsubscript{1} (PD20FEV\textsubscript{1}) was positively correlated with prechallenge FEV\textsubscript{1}, percent predicted (Spearman correlation \(r = 0.35, p < 0.0001\)). The fifth percentile of PD20FEV\textsubscript{1}, chosen as an estimate of the lower limit of the normal range, varied with the level of prechallenge FEV\textsubscript{1}. When applied to a larger sample of 838 men with normal pulmonary function, the use of FEV\textsubscript{1}-specific cut-off values to separate "normal" from "abnormal" PD20FEV\textsubscript{1} did not improve the sensitivity or specificity of methacholine challenge as a test for questionnaire-reported asthma or wheezing. These data provide lower limits of normal PD20FEV\textsubscript{1}, which are specific for a subject's prechallenge FEV\textsubscript{1}; however, these FEV\textsubscript{1}-specific lower limits of normal PD20FEV\textsubscript{1} provided no greater sensitivity and specificity for detecting asthma and wheezing than did a single lower limit of normal PD20FEV\textsubscript{1} for all subjects.

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Methacholine challenge testing is currently being used as a diagnostic test for a variety of clinical indications. In patients presenting with chronic cough of uncertain cause, challenge testing has been advocated as a means to determine whether the cough is associated with airway hyperresponsiveness ("cough variant asthma") and likely to respond to bronchodilator therapy.\textsuperscript{1} Methacholine challenge testing also has a role in the evaluation of workers with possible occupational asthma for purposes of diagnosis or disability determination.\textsuperscript{2,3}

Clinical interpretation of challenge tests performed for these indications requires the establishment of a normal range of challenge test results. Distinguishing normal from abnormal airway responsiveness is complicated by the overlapping ranges of responsiveness displayed by normal (asymptomatic, nonasthmatic) and abnormal (asthmatic) subjects.\textsuperscript{4,5} Such overlap is a feature of most measurements used as diagnostic tests, and the selection of a "cut-off" between normal and abnormal generally involves a tradeoff between diagnostic sensitivity and specificity.

Another aspect of bronchial challenge testing that adds complexity to the establishment of a normal range is the relationship between measurements of airway responsiveness and the level of pulmonary function prior to bronchial challenge. Responsiveness to methacholine or histamine has been observed to be inversely related to level of pulmonary function (\(te\), greater responsiveness observed in persons with lower levels of pulmonary function) in population samples\textsuperscript{6-12} and among patients with COPD.\textsuperscript{13-17} Some reports have indicated a similar relationship among subjects with asthma,\textsuperscript{18,19} but other investigators have observed no relationship between responsiveness and prechallenge pulmonary function among asthmatics.\textsuperscript{16,20}

Previous descriptions of the relationship between airway responsiveness and prechallenge level of pulmonary function have not provided normal ranges of methacholine airway responsiveness for specific levels of pulmonary function, limiting the clinical usefulness of these reports. In this study, we examined the distribution of methacholine airway responsiveness according to level of prechallenge FEV\textsubscript{1} in a sample of asymptomatic middle-aged and elderly men with normal pulmonary function. The use of FEV\textsubscript{1}-specific criteria for abnormal PD20FEV\textsubscript{1}, was compared with the use of a single lower limit of normal PD20FEV\textsubscript{1}, in terms of sensitivity and specificity for detecting...
questionnaire-reported asthma and wheezing in a larger sample that included men with respiratory symptoms.

**METHODS**

**Sample**

The Normative Aging Study is a longitudinal cohort study of health and aging established at the Veterans Administration's Outpatient Clinic in Boston in 1963. Volunteers were recruited by advertisement and accepted into the study only if initial health screening revealed no evidence of chronic diseases including asthma, sinusitis, hypertension, or heart disease. The initial cohort consisted of 2,280 men between the ages of 21 and 80 years. Since entry into the study, subjects have reported periodically for comprehensive health assessment, including medical history, physical examination, spirometry, electrocardiogram, chest radiograph, and blood tests. Beginning in 1984, subjects have completed respiratory illness and symptom questionnaires and have undergone methacholine challenge testing every 3 years. As described in detail below, the present analysis is based on 1,410 subjects who reported for at least one examination between April 1984 and June 1990.

**Questionnaire**

A self-completed respiratory illness and symptom questionnaire adapted from the ATS-DLD questionnaire was completed by all subjects within 1 week prior to methacholine challenge testing. "Current asthma" was defined as an affirmative response to the questions "have you ever had asthma?" and "do you still have it?" "Former asthma" was defined as an affirmative response to the former question with a negative response to the latter question. "Episodic wheeze with dyspnea" was defined as an affirmative response to the question "have you ever had attacks of wheezing with shortness of breath?" "Persistent wheeze" was defined as an affirmative response to the question "have you ever had wheezing in your chest?" which included either "most days and nights" or "both with colds" and "occasionally apart from colds." A subject was considered asymptomatic if he denied any history of asthma, wheezing, usual cough, usual phlegm, chronic bronchitis, or shortness of breath sufficient to cause stopping to catch breath. A subject was considered a lifetime nonsmoker if he denied smoking at least one cigarette per day at present and had smoked less than 20 packs in his lifetime.

**Spirometry**

Baseline (prechallenge) spirometry was performed in the standing position, wearing noseclips, with the use of a water-filled survey spirometer (Collins 8-L, Warren Collins, Inc, Braintree, Mass) in accordance with published guidelines. The FVC and FEV1 corrected to BTPS, were calculated by a microprocessor (Eagle II, Warren Collins Inc, Braintree, Mass).

**Methacholine Challenge**

Methacholine inhalation challenge testing was performed as previously described.4 Solutions were aerosolized by a nebulizer (DeVilbiss 646) powered by an air compressor (DeVilbiss). All inhalations were 6-s vital capacity inhalations followed by 2 s of breathholding. Second were counted aloud from a digital stop-watch after careful instruction and practice. Inhalations were done seated and wearing noseclips. The protocol consisted of six levels, which varied in the number of inhalations and the methacholine concentration as follows: five inhalations at 0 mg/ml (phenol-buffered saline solution alone), one inhalation at 1 mg/ml, one inhalation at 5 mg/ml, four inhalations at 5 mg/ml, one inhalation at 25 mg/ml, and four inhalations at 25 mg/ml. Inhalation series were performed at 5-min intervals. After each inhalation level, spirometry was performed as described above at 30, 90, and 180 s. If the first two spirometers at each level were consistent (FVC and FEV1 within 5 percent), then the higher FEV1 value of these two was chosen for analysis. Otherwise the higher FEV1 value from the two most consistent acceptable spirometers was used. Testing was terminated when a 20 percent decline in FEV1 occurred or at the end of the protocol. Nebulizer output was determined by weighing the nebulizer before and after a series of inhalations performed in the same manner as they would be performed during the challenge test. These output measurements were repeated periodically and remained consistent throughout the interval during which these data were collected. The mean nebulizer output was 0.0645 ml per inhalation, indicating cumulative methacholine doses of 0 (saline solution), 0.330, 1.98, 8.58, 16.8, and 49.8 μmol of methacholine at the six respective levels of the protocol.

The PD20FEV1 was calculated by linear interpolation.4 Subjects who did not experience a 20 percent or greater fall in FEV1 by the end of the protocol did not have a calculable value of PD20FEV1. For purposes of calculating the fifth percentile of PD20FEV1 in the sample, these subjects were considered to have PD20FEV1 greater than 49.8.

**Analysis**

Multiple linear regression, Spearman's correlation, and calculation of fifth and tenth percentiles were performed using a standard statistical software package (SAS, Inc, Carey, NC). Sensitivity is defined as the number of true positive test results divided by the sum of true positives plus false negatives. Specificity is defined as the number of true negative test results divided by the sum of true negatives plus false positives.

**RESULTS**

Between April 1984 and June 1990, 1,410 men reported for at least one examination. Two hundred fifteen subjects were lifetime nonsmokers and denied any history of respiratory illness or symptoms. Regression analysis restricted to these 215 subjects yielded the following equations:

- Predicted FVC (L) = -4.46 + 0.155 height (in) - 0.0324 age (yrs)
- Predicted FEV1 (L) = -1.34 + 0.0076 height (in) - 0.0326 age (yrs)
- Predicted FEV1/FVC ratio (%) = 137 - 0.655 height (in) - 0.184 age (yrs)

The distribution of FEV1 (percent predicted) and FEV1/FVC ratio (percent predicted) among these 215 men revealed that only 5 percent had values less than 77 percent predicted and 86 percent predicted, respectively. These values were chosen as the lower limits of normal FEV1 and FEV1/FVC ratio for this analysis.

Of the 1,410 subjects who were examined during this time period, 105 men were ineligible for methacholine challenge testing because of cardiovascular disease or prechallenge FEV1, less than 60 percent of predicted. Of the 1,305 eligible subjects, 1,024 (78 percent) performed at least one acceptable methacholine challenge. Acceptable challenge data were not obtained from the remaining 281 subjects because of unacceptable subject performance of aerosol inhalations or forced expiratory maneuvers (n = 40).
Table 1—Characteristics of 1,410 Male Normative Aging Study Subjects*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Included in Analysis (n = 838)</th>
<th>Excluded from Analysis† (n = 572)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>60.5 (7.7)</td>
<td>61.3 (8.1)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>172.0 (6.6)</td>
<td>172.0 (6.4)</td>
</tr>
<tr>
<td>FVC, percent predicted†</td>
<td>99.4 (12.4)</td>
<td>83.4 (17.7)</td>
</tr>
<tr>
<td>FEV₁, percent predicted†</td>
<td>98.9 (11.9)</td>
<td>89.1 (16.3)</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>0.80 (.05)</td>
<td>0.75 (.09)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>9.4</td>
<td>22.7</td>
</tr>
<tr>
<td>Former</td>
<td>53.0</td>
<td>53.7</td>
</tr>
<tr>
<td>Never</td>
<td>37.6</td>
<td>23.6</td>
</tr>
<tr>
<td>Asthma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, still</td>
<td>1.1</td>
<td>1.9</td>
</tr>
<tr>
<td>Yes, in past</td>
<td>2.3</td>
<td>3.5</td>
</tr>
<tr>
<td>Never</td>
<td>96.7</td>
<td>93.5</td>
</tr>
<tr>
<td>Missing</td>
<td>—</td>
<td>1.0</td>
</tr>
<tr>
<td>Persistent wheeze</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5.1</td>
<td>12.8</td>
</tr>
<tr>
<td>No</td>
<td>94.7</td>
<td>85.8</td>
</tr>
<tr>
<td>Missing</td>
<td>0.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Ever had attack of wheeze and dyspnea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3.6</td>
<td>7.9</td>
</tr>
<tr>
<td>No</td>
<td>94.7</td>
<td>89.3</td>
</tr>
<tr>
<td>Missing</td>
<td>1.7</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*Values are mean (SD) or percent.
†See text.
‡Predicted values derived from regression equations based on 215 asymptomatic never smokers in the Normative Aging Study.

refusal to undergo or to complete challenge (n = 224), physician decision to stop test because of minor side effects or discomfort (n = 7), or technician error and miscellaneous other reasons (n = 10). Of the 1,024 subjects with acceptable methacholine challenge data, 841 had a FEV₁ greater than or equal to 77 percent predicted and a FEV₁/FVC ratio greater than or equal to 86 percent predicted, i.e., in the normal range as defined above. Three subjects experienced a 20 percent or greater drop in FEV₁ after inhaling diluent (phenol-buffered saline solution) and are excluded from further analyses. Characteristics of the 838 subjects included in further analyses, as well as those excluded, are given in Table 1. As expected on the basis of the exclusion criteria employed, the excluded subjects have lower pulmonary function and higher prevalences of smoking and respiratory symptoms than the included subjects.

Among the 838 subjects with normal FEV₁ and FEV₁/FVC ratio, 547 (65 percent) denied any history of respiratory illness or chronic respiratory symptoms. The PD20FEV₁ was significantly correlated with prechallenge FEV₁ (percent predicted) among these 547 subjects (Spearman correlation coefficient = 0.35, p < 0.0001). Among these 547 subjects, the fifth percentile of PD20FEV₁ (i.e., the value of PD20FEV₁ which has 5 percent of the measurements below it) was 8.3 μmol.

The 547 asymptomatic subjects with normal pulmonary function were grouped according to quintile of prechallenge FEV₁ (percent predicted), and the fifth percentiles of PD20FEV₁ within each quintile of FEV₁ were calculated (Table 2). The fifth percentile of methacholine PD20FEV₁, increased with increasing quintile of prechallenge FEV₁. This relationship is shown graphically in Figure 1, in which the fifth percentile of PD20FEV₁ for each quintile is plotted against the median FEV₁ for that quintile. Restricting analysis to a subgroup of 157 lifetime nonsmokers yielded similar values for the fifth percentiles of PD20FEV₁, for both overall and FEV₁-specific percentiles (data not shown).

The overall fifth percentile of PD20FEV₁ (8.3 μmol) and the FEV₁-specific fifth percentiles of PD20FEV₁ (as indicated in Table 2) respectively defined the 26 (4.8 percent) and 27 (4.9 percent) most responsive subjects among the 547 asymptomatic men. Seventeen (3.1 percent) men were classified as hyperresponders by both approaches, but 19 (3.5 percent) men were classified differently by these alternative definitions of hyperresponsiveness.

These alternative definitions of hyperresponsiveness were compared in terms of sensitivity and specificity for detecting questionnaire-reported asthma and wheezing among all 838 subjects with normal FEV₁ and FEV₁/FVC ratio. There was no appreciable differ-
ence in sensitivity and specificity between using the overall fifth percentile as opposed to the FEV₁-specific fifth percentiles as the lower limit of normal PD20FEV₁ (Table 3). A parallel analysis, which is not shown, used definitions of hyperresponsiveness based on the tenth rather than fifth percentile of PD20FEV₁ and led to the same conclusion: FEV₁-specific lower limits of normal PD20FEV₁ provided no greater sensitivity and specificity for detecting asthma and wheezing than did a single lower limit of normal PD20FEV₁ for all subjects.

**Discussion**

We observed that methacholine PD20FEV₁ was positively correlated with prechallenge FEV₁ in a sample of middle-aged and elderly men who denied respiratory symptoms or illnesses and who had normal prechallenge spirometry. When these healthy subjects were grouped into quintiles according to prechallenge FEV₁, the lower limit of normal PD20FEV₁, defined arbitrarily as the fifth percentile of PD20FEV₁, varied across quintiles. When applied to the overall sample, which included men with respiratory disease, the use of FEV₁-specific cut-off values to separate "normal" from "abnormal" PD20FEV₁ did not improve the sensitivity or specificity of methacholine challenge as a test for questionnaire-reported asthma or wheezing. A number of mechanisms have been proposed as the basis for the relationship between nonspecific airway responsiveness and prechallenge pulmonary function.²⁴⁻²⁶ Because resistance to flow through a tube is inversely proportional to the radius to the fourth power, a given degree of bronchoconstrictor-induced circumferential airway narrowing can be expected to cause a proportionally larger increase in airway resistance in a narrow airway than in a wider airway. Intersubject differences in the distribution of resistance along the bronchial tree may influence both the pattern of aerosol deposition and the relative impact on total airway resistance of bronchoconstriction at particular sites such as the peripheral airways. Airway hyperresponsiveness may result in heightened bronchomotor tone prior to challenge and thereby be correlated with reduced prechallenge pulmonary function. The convention of expressing responsiveness in terms of percentage of change in FEV₁ (eg, PD20FEV₁) imposes a mathematical relationship between FEV₁ and such measures of responsiveness. In subjects with lung disease, additional mechanisms may contribute to the direct relationship between prechallenge FEV₁ and PD20FEV₁. Inflamed, edematous bronchial mucosa can be expected to occupy a greater proportion of airway lumen than normal mucosa for a given degree of bronchial smooth muscle narrowing, decreasing both FEV₁ and PD20FEV₁. Inflammation and emphysema may also decrease the mechanical load against which bronchial smooth muscle must contract, leading to greater responsiveness in persons with low prechallenge pulmonary function.²⁶

An alternative, or additional, hypothetical explanation for the cross-sectional relationship between FEV₁ and PD20FEV₁ is that airway hyperresponsiveness precedes and is a risk factor for impaired growth or accelerated decline of pulmonary function.²⁷ This theory, often considered a central part of the "Dutch hypothesis" first proposed by Van der Lende and coworkers,²⁸ is the subject of ongoing investigation in prospective longitudinal studies. Although the relationship of prechallenge pulmo-

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**Table 2—Fifth Percentile of PD20FEV₁ in Relation to Prechallenge FEV₁ (Percent Predicted) Among 547 Asymptomatic Normative Aging Study Participants With Normal Prechallenge FEV₁ and FEV₁/FVC Ratio**

<table>
<thead>
<tr>
<th>FEV₁, % pred (Quintile)</th>
<th>n</th>
<th>FEV₁, % pred (Range)*</th>
<th>PD20FEV₁, Fifth percentile µmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>109</td>
<td>77.1-89.2</td>
<td>4.2</td>
</tr>
<tr>
<td>2</td>
<td>110</td>
<td>89.2-96.1</td>
<td>8.2</td>
</tr>
<tr>
<td>3</td>
<td>109</td>
<td>96.2-102.8</td>
<td>11.5</td>
</tr>
<tr>
<td>4</td>
<td>110</td>
<td>102.9-110.4</td>
<td>25.1</td>
</tr>
<tr>
<td>5</td>
<td>109</td>
<td>110.4-140.3</td>
<td>39.0</td>
</tr>
</tbody>
</table>

*Some ranges overlap due to rounding.

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**Table 3—Sensitivity and Specificity of Alternative Fifth Percentile-Based Definitions of Hyperresponsiveness for Detecting Asthma and Wheezing Among 838 Normative Aging Study Participants With Normal Prechallenge Pulmonary Function*†**

<table>
<thead>
<tr>
<th>Symptom</th>
<th>PD20FEV₁ &lt; 8.3 µmol</th>
<th>PD20FEV₁ &lt; FEV₁-Specific Fifth Percentile†</th>
<th>PD20FEV₁ &lt; FEV₁-Specific Fifth Percentile†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity, %</td>
<td>Specificity, %</td>
<td>Sensitivity, %</td>
</tr>
<tr>
<td>Current asthma</td>
<td>44</td>
<td>93</td>
<td>56</td>
</tr>
<tr>
<td>Ever asthma</td>
<td>32</td>
<td>94</td>
<td>36</td>
</tr>
<tr>
<td>Episodic Wheeze and dyspnea</td>
<td>30</td>
<td>94</td>
<td>27</td>
</tr>
<tr>
<td>Persistent wheeze</td>
<td>14</td>
<td>93</td>
<td>16</td>
</tr>
</tbody>
</table>

*As defined in text.
†FEV₁-specific fifth percentile PD20FEV₁ values are indicated in Table 2.
nary function to nonspecific airway responsiveness is consistent in a number of population studies.\textsuperscript{6-12} Prechallenge FEV\textsubscript{1} appears to explain only a small proportion of the variance in PD\textsubscript{2}OFEV\textsubscript{1}, seen in the population. This is not surprising inasmuch as the control of bronchomotor tone involves a complex, integrated physiologic system, and prechallenge pulmonary function is undoubtedly only one of many factors that influence this system.

The failure of FEV\textsubscript{1}-specific normal ranges of PD\textsubscript{2}OFEV\textsubscript{1} to improve sensitivity or specificity in detecting questionnaire-reported asthma or wheezing has several possible explanations. Measurements of airway responsiveness are imperfect at discriminating between asthmatics and nonasthmatics in population samples,\textsuperscript{5,29,30} and altering the criteria for an abnormal challenge test may have little impact relative to other sources of imprecision and inaccuracy. Furthermore, adjusting the "normal range" of PD\textsubscript{2}OFEV\textsubscript{1}, to remove the influence of level of FEV\textsubscript{1} on PD\textsubscript{2}OFEV\textsubscript{1}, will not necessarily improve predictive power if both level of pulmonary function and degree of airway responsiveness contribute to the occurrence of wheezing symptoms and to the likelihood of a clinical diagnosis of asthma in this age group.

The fifth percentile values chosen as the lower limits of normal PD\textsubscript{2}OFEV\textsubscript{1} in this analysis were derived from a sample that included both nonsmokers and smokers. The relatively small number of lifetime nonsmokers with no symptoms and normal pulmonary function (n = 157) favored using the data of all 547 asymptomatic men with normal lung function, regardless of smoking history, to define the lower limits of normal PD\textsubscript{2}OFEV\textsubscript{1}. A separate analysis restricted to the 157 never smokers revealed a similar distribution of PD\textsubscript{2}OFEV\textsubscript{1}, with respect to prechallenge FEV\textsubscript{1}. Furthermore, among Normative Aging Study subjects who deny respiratory symptoms and who have normal pulmonary function, methacholine responsiveness is not significantly related to smoking status (unpublished). We have previously reported that current smoking is associated with slightly greater methacholine airway responsiveness among atopic subjects,\textsuperscript{31} however, that analysis was not restricted to asymptomatic subjects with normal lung function.

In previous reports we have utilized dose-response slope\textsuperscript{32} to analyze methacholine responsiveness data. This report focuses on PD\textsubscript{2}OFEV\textsubscript{1}, because it is the index of responsiveness used by most clinical laboratories employing methacholine challenge as a diagnostic test. A parallel analysis using dose-response slope yielded results similar to those reported above.

The Normative Aging Study cohort is an excellent source of normative data, but it has limitations in terms of evaluating the accuracy of a diagnostic test. Subjects were tested as part of their ongoing participation in the Study and are not directly comparable to patients presenting for evaluation of respiratory symptoms. Asthma and wheeze reported on questionnaire may have different implications from those prompting a visit to a physician. A more appropriate sample with which to compare the accuracy of conventional and FEV\textsubscript{1}-specific normal ranges for PD\textsubscript{2}OFEV\textsubscript{1}, would be, for example, a group of patients presenting with persisting unexplained cough in which alternative criteria for abnormal responsiveness could be compared in terms of ability to predict a therapeutic response to inhaled bronchodilator or corticosteroid therapy.

In summary, these data indicate that the "normal range" of methacholine PD\textsubscript{2}OFEV\textsubscript{1}, varies with prechallenge FEV\textsubscript{1}, even among persons who deny respiratory symptoms or illnesses and who have normal pulmonary function. Using FEV\textsubscript{1}-specific criteria for defining the lower limit of "normal" PD\textsubscript{2}OFEV\textsubscript{1}, however, did not enhance the sensitivity and specificity of methacholine challenge testing for detecting asthma and wheezing in Normative Aging Study subjects.

\textbf{References}

5. Pattemore PK, Asher MI, Harrison AC, Mitchell EA, Rea HH, Stewart AW. The interrelationship among bronchial hyperresponsiveness, the diagnosis of asthma, and asthma symptoms. Am Rev Respir Dis 1990; 142:549-54

Normal Range of Methacholine Responsiveness (O'Connor, Sparrow, Weiss)