The Flaccid Lung Syndrome and α₁-Protease Inhibitor Deficiency

Kees D. Laros, M.D., Ph.D.;* Izak Biemond, M.D.;† and Eduard C. Klasen, Ph.D.‡

We examined breathing mechanics and α₁,PI deficiency in 1,850 unrelated male subjects with various lung complaints. The loss in lung elasticity appeared to be significantly more pronounced in ZZ individuals as compared to MM, MS and also MZ individuals. The MZ group did not differ significantly in this respect from MM individuals. This implies that the excess risk of developing a flaccid lung (C > 1 kPa⁻¹) due to the partial α₁-antitrypsin deficiency is negligible. PI MZ and PI ZZ frequencies are significantly higher in the population with flaccid lung compared to control subjects. Furthermore, it was found that the increase in residual volume in smokers is independent of the PI type.

Several tissues and body fluids contain protease inhibitors to provide protection against proteolytic activity. Hereditary deficiency of one or more inhibitors causes a protease-antiprotease imbalance which may lead to structural damage. This so-called protease-antiprotease theory was first formulated following the observation that approximately 80 percent of the PI ZZ individuals develop panlobular emphysema. The extent of the risk for PI MZ heterozygotes to develop chronic obstructive pulmonary disease (COPD) has been the subject of considerable controversy. Assessment of such risk is important since MZ individuals constitute 3-5 percent of most Caucasian populations. In an attempt to solve this problem,²,³ investigations were based upon two approaches: 1) determination of the prevalence of heterozygotes among patients with COPD, and 2) whether or not heterozygotic subjects had COPD more often than control subjects.

One of the main problems in comparing the results of the various reported studies has been the lack of consistency in criteria used to define COPD. For this reason, we decided to study a well-defined subgroup of the COPD population, namely: patients who suffered from a so-called “flaccid lung.” The term flaccid lung pertains to a loss of elasticity of the lung parenchyma defined as a high compliance in relation to the actual functional residual capacity.⁴ Individuals were considered to have a flaccid lung when the volume compliance is >1.0 kPa⁻¹ compared to the volume compliance of normal lung elasticity which is between 0.5 and 0.8 kPa⁻¹. Flaccid lung can be found in patients with a large vital capacity (>120 percent of the predicted value), in patients with spontaneous pneumothorax, giant bullae or lung emphysema. If the protease-antiprotease imbalance does play a role in the development of a flaccid lung, one would expect a significantly higher incidence of homozygous and heterozygous α₁-PI deficient individuals among the population with flaccid lung, compared to normal individuals. To test this hypothesis, 1,850 unrelated male patients with COPD were studied for α₁-PI levels and phenotypes, as well as for lung function parameters and smoking habits.

Subjects and Methods

Subjects

In the period 1979-1984, 1,850 unrelated male patients with COPD, visiting the outpatient lung department of St Antonius Hospital, Utrecht/Nieuwegein, The Netherlands, were examined for pulmonary function including breathing mechanics and blood gases both at rest and during exercise.

We have investigated 82 percent of the patients for several years, (mean observation time 7.4 years, range 1-27 years); 75 percent of them had had one or more clinical check-ups. The minimum observation time in all cases was one year.

The healthy control subjects were a random sample of Dutch blood donors.⁶ The patients were then subdivided into groups, according to their volume compliance: 1) those with a normal or increased lung elasticity (internal controls), 2) those with a decreased lung elasticity (the flaccid lung group), and 3) an intermediate group of patients. The volume compliance, (the ratio of quasi-static lung compliance: functional residual capacity), was used as the discriminating factor. The patient's family, social and work history; smoking habits, chest x-ray film studies, lung function test results (including esophageal pressure-volume studies), and blood gas levels both at rest and during exercise were recorded.

Methods

Sera were stored in batches at −30°C immediately after collection. PI phenotyping was carried out using isoelectric focusing with restricted pH range carrier ampholytes, a separator, and a highly crosslinked gel.⁷ The level of α₁-PI was determined by single radial immunodiffusion. Aliquots of pooled sera from 326 healthy blood donors served as a reference standard to which a value of 100 percent was assigned. Spirograms were made using water-sealed spirometers. The functional residual capacity (FRC) was measured with the helium dilution method, using a 33 percent initial helium concentration in a 40 percent oxygen, 27 percent nitrogen mixture.
The coefficient of variation for repeated FRC measurements in normal subjects was 4.2 percent.

Lung compliance ($C_L$) was measured with a Latex balloon 10 cm in length. In each instance, a pressure-volume plot of the balloon was made in air to test the standard quality, and the inter-balloon comparability. The balloon was placed in the esophagus under x-ray guidance with its lower tip 4 cm below the main bifurcation. A pressure-volume plot of the balloon in situ was made, and in line with this plot, the filling of the balloon was fixed at mid-plateau. In each patient, three quasi-static expiratory PV curves were produced. The quasi-static lung compliance ($C_L$) was calculated from the best fitting line going from FRC to FRC + 0.5 liter. Intra-patient comparability was good, though dependent on the correct positioning of body and head and the balloon. Standard error in 50 normal subjects was ±0.104 L/kPa. Airway resistance ($R$) was calculated from the viscous work ($W$) as derived from the surface area of a tidal pressure-volume loop ($P_V$) according to the formula:

$$R = 0.2\frac{W}{V_T f}$$

($W$ in J, $V_T$ in L and $f$ in Hz)

In each patient the measurements of $C_L$, $R$ and lung volumes including FRC were made before and after administration of a bronchodilator drug at the same session. The data given in the tables are those obtained after bronchodilation. The following lung function parameters were used in this study: the inspiratory vital capacity ($V_C, L_{BTPS}$) as a percentage of the predicted vital capacity ($V_C$); the volumic or specific compliance as the quotient of lung compliance and the actual functional residual capacity ($C_{V,F} = C_L/FRC$); the ratio between the forced one second expiratory volume ($FEV_1$) and the one second inspiratory volume ($FIV_1$); the residual volume ($R,L_{BTPS}$) as a percentage of the total lung capacity (TLC).

Pronounced loss in lung elasticity is characterized by a significant decrease in $FEV_1$, whereas the $FIV_1$ stays normal, leading to a definite decrease in the $FEV_1/FIV_1$ ratio, normal range 0.75-0.85. Age, height and body weight, as well as the smoking habits at the last check-up were used. All data were coded and computerized for analysis using the statistical program package SPSS.

## Results

In the total population studied, the following phenotypes were found (exact numbers are available at request): MM, MI, MF, MX, FF, FS, MS, SS, MZ, FZ, SZ, ZO, ZZ, MO and a rare PI type (SWFIN). In order to be able to perform a reliable statistical analysis, the rare phenotypes were excluded and our study was focused on the remaining 1716 MM, MS, MZ and ZZ individuals.

### Table 1—PI Phenotype Frequencies in Relation to Lung Elasticity

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>Controls</th>
<th>Healthy controls</th>
<th>Internal controls</th>
<th>Intermediate</th>
<th>Flaccid lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_L$ (kPa$^{-1}$)</td>
<td>MM</td>
<td>MS</td>
<td>MZ</td>
<td>ZZ</td>
</tr>
<tr>
<td>Healthy controls</td>
<td></td>
<td>92.1</td>
<td>5.4</td>
<td>2.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Internal controls</td>
<td>&lt;0.8</td>
<td>90.4</td>
<td>6.1</td>
<td>3.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Intermediate</td>
<td>0.8&lt;-&lt;1.0</td>
<td>90.4</td>
<td>6.3</td>
<td>3.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Flaccid lung</td>
<td>&gt;1.0</td>
<td>86.4</td>
<td>6.8</td>
<td>5.3</td>
<td>1.5</td>
</tr>
</tbody>
</table>

*Note: *$C_L = C_{V,F}$/FRC = volumic compliance

In the period 1979-1984, 1,850 random male patients visiting the outpatient department were screened for breathing mechanics by the esophageal balloon method. The 1,716 patients with PI types MM, MS, MZ and ZZ were subdivided into four groups according to their volume compliance ($C_V$).

The 384 patients with normal lung elasticity and the 75 with increased lung elasticity were used as internal control subjects (26.7 percent of the total group). In 333 patients (19.4 percent of the total group), the $C_V$ values were in between those of the normal group and of the group with a definite loss in lung elasticity; the intermediate group. The “flaccid lung” group was composed of 465 patients with moderate and 459 patients with an exceptional loss in lung elasticity (ie, 53.8 percent of the total group).

Table 1 represents the PI phenotype distribution for both healthy control subjects and COPD patients in relation to lung elasticity. It is clear that the vast majority of the ZZ individuals (88 percent), are present among the group with flaccid lung, whereas the heterozygotes MZ are more evenly distributed: 65 percent of the MZ individuals are present in the group with flaccid lung.

The demographic characteristics and lung parameters of the patients with flaccid lung are shown in Table 2. A new and surprising finding is that the ZZ individuals are significantly taller than the MM individuals ($p < 0.05$). Furthermore, both the $FEV_1/V_C$ and $FEV_1/FIV_1$ ratio differ significantly in ZZ individuals compared to the MM, MZ and MS individuals in

### Table 2—Characteristics of 924 FLS Patients; ($C_V > 1$ kPa$^{-1}$) in Relation to Phenotypes

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>MM</th>
<th>MS</th>
<th>MZ</th>
<th>ZZ</th>
<th>Analysis of Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>798</td>
<td>63</td>
<td>49</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>54.1</td>
<td>13.9</td>
<td>54.9</td>
<td>12.3</td>
<td>52.9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.1</td>
<td>7.4</td>
<td>177.2</td>
<td>7.1</td>
<td>178.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.6</td>
<td>11.3</td>
<td>76.1</td>
<td>10.9</td>
<td>76.7</td>
</tr>
<tr>
<td>RV/TLC (%)</td>
<td>35.6</td>
<td>10.0</td>
<td>36.2</td>
<td>11.3</td>
<td>35.2</td>
</tr>
<tr>
<td>FEV$_1$/VC (%)</td>
<td>55.5</td>
<td>16.6</td>
<td>53.6</td>
<td>17.7</td>
<td>53.8</td>
</tr>
<tr>
<td>FEV$_1$/FIV$_1$</td>
<td>0.62</td>
<td>0.18</td>
<td>0.60</td>
<td>0.18</td>
<td>0.61</td>
</tr>
<tr>
<td>VCF/VCp (%)</td>
<td>100.3</td>
<td>19.3</td>
<td>100.4</td>
<td>20.6</td>
<td>104.6</td>
</tr>
<tr>
<td>RR(kPa/L/A)</td>
<td>0.35</td>
<td>0.25</td>
<td>0.40</td>
<td>0.27</td>
<td>0.35</td>
</tr>
</tbody>
</table>

The Flaccid Lung Syndrome (Laros, Biemond, Klasen)
this group with flaccid lung (p < 0.01, and p < 0.005, respectively). This indicates that the loss in lung elasticity is significantly more pronounced in ZZ individuals than in MM, MS and MZ individuals.

Smokers and nonsmokers are uniformly distributed over the different phenotype groups. In order to estimate the influence of smoking as a single predisposing factor on the development of FLS, a comparison was made between PI MM smokers and PI MM nonsmokers both with and without the flaccid lung syndrome (Table 3). Using the Cv for the diagnosis of FLS the risk of MM-smokers to develop a flaccid lung appeared to be 1.6 times the risk of MM nonsmokers (relative risk of 1.6). The calculated risk for MZ smokers towards nonsmokers also equals 1.6, whereas ZZ smokers showed a relative risk of 6.0 compared to ZZ nonsmokers.

The mean residual volume, RV%TC, for MM, MZ and ZZ smokers, as well as nonsmokers is shown in Table 4. Within the MM and MZ phenotype groups, the mean residual volume is significantly larger among the smokers than among the nonsmokers (p < 0.01). However, the mean residual volumes of MM, MZ and ZZ smokers are comparable. This means that the increase in residual volume in smokers is independent of the PI type.

For the diagnosis of FLS, the criterion used is volume compliance (Cv). However, as is shown by our data, the FEV/FIV ratio <0.75 also accounts for individuals with a flaccid lung (Table 5). In this table, the mean Cv of the total population studied is shown, subdivided in the two FEV/FIV groups (>0.75 and <0.75) according to PI phenotypes. There was no significant difference between the MZ and MM population on the one hand and the MS and MM population on the other. However, the ZZ group with a FEV/FIV ratio <0.75 does differ significantly from all other groups (p <0.001). On the basis of these data, we have calculated that 40 percent of the total population studied exhibits FLS according to both the Cv and the FEV/FIV ratio. When considering those FLS patients diagnosed by Cv alone, 75 percent had a FEV/FIV ratio <0.75. This proves that a good correlation exists between the Cv and the FEV/FIV ratio.

As the average α1-PI concentration in the different phenotype groups in the flaccid lung population did not differ significantly from that in either the internal controls or in that of the healthy control population, an acute phase reaction was not likely to be present among the patients studied.

**DISCUSSION**

In clinical practice, the post-mortem histologic features of loss in lung elasticity as a cause of breathlessness are not available. The diagnosis in life depends upon the clinical picture, the chest radiographic findings and on pathophysiologic parameters. Emphysema is a term used to describe the anatomic changes in the lung observed by resection of lung tissue or at autopsy. During life, only indirect indications for loss of structure leading to flaccidity of the lung parenchyma can be obtained. In our experience the most important combination of lung function tests indicating loss in lung elasticity is: 1) ratio between forced expiratory volume change (FEV) and forced inspiratory volume change (FIV); in one second. Loss in lung elasticity produces air flow limitation mainly on expiration. The shape of the FIV curve, as of the inspiratory flow volume curve, is similar to a normal curve (Fig 1 and 2); 2) total lung capacity (TLC) and functional residual capacity (FRC). A decrease in elastic recoil of the lung,
leading to disturbed expiration with normal inspiration, will, due to air trapping, shift the breathing level towards the inspiratory side, thus leading to increased FRC and TLC values; 3) volume “specific” compliance is defined as $C_L/FRC$. An early and sensitive measurement to detect loss in lung elasticity is, in our experience, the increase in specific lung compliance. It is the most direct indication that the elastic recoil of the lung is decreased. It is because of a missing histologic substrate during life, and the fact that evidence for loss of elastic recoil can be obtained by the function tests mentioned above, that we have chosen for the clinically accessible term “flaccid lung” rather than for the, in practice, inaccessible histologic term emphysema. A flaccid lung is a lung in which signs of loss of elastic recoil can be demonstrated by the use of lung function tests.

We have clearly demonstrated significant differences in lung elasticity between ZZ individuals on the one hand and MZ, MS and MM individuals on the other. The MZ group did, however, not differ significantly in this respect from MM individuals. This leads us to conclude that PI MZ individuals do not develop a flaccid lung more often or sooner than do PI MM individuals; thus, the excess risk due to the deficiency is negligible. The excess influence of smoking on the development of a flaccid lung is most striking in the case of ZZ individuals (relative risk 6.0). Our findings are in agreement with those of other investigators.\(^\text{11,12}\)

In a recent collaborative study to assess the risk of
lung disease in a large number of individuals with PI MZ. It was concluded that the MZ phenotype alone carries no greater risk of developing lung disease than MM phenotype. Other studies have shown a significantly higher number of MZ individuals among COPD patients as compared to control subjects. The risk of developing COPD for PI MZ individuals in these studies was on average three times higher than that of the normal population. These differing conclusions are due, in our opinion, to differences in the number of individuals studied and to the different criteria to define COPD. For instance, in contrast to the present study, most investigators did not measure compliance which is the only method of measuring lung elasticity. We conclude that if a PI MZ individual does develop a flaccid lung, the excess risk due to the deficiency is negligible compared to MM individuals and is highly influenced or modified by other factors, possibly environmental (smoking, genetic). This is in agreement with the findings of one of the few investigators who did measure the compliance and concluded that the risk of disablement from chronic lung disease appears to be only slightly enhanced by intermediate α1AT deficiency. In view of the development of a reliable substitution therapy for α1-antitrypsin deficient individuals, the early recognition of ZZ homozygotes will become more and more important in the future. For this reason we have started collecting and studying families of MZ and ZZ propositi.

ACKNOWLEDGMENTS: The authors thank Mrs. E. van der Kooij-Meijls for technical assistance and Mrs. A. van der Ende-Roos for preparation of the manuscript. This work was supported by the Netherlands Asthma Foundation, Grant No. 80.33.

REFERENCES
5 Klasen EC, Bos A, Simmelink HD. PI (α1-antitrypsin) subtypes: frequency of PI*M4 in several populations. Hum Genet 1982; 62:139-41
6 Klasen EC, Rigotti A. Isoelectric focusing of α1-antitrypsin (PI) using restricted pH-range carrier ampholytes in combination with a highly cross-linked gel and a separator. Electrophoresis 1982; 3:168-71
8 Douma JH. Reynolds similarity law applied to airway resistance. Bull Eur Physiopathol Respir 1969; 5:385-95
10 Klasen EC, Laros CD, Brocker-Vriends A, Frants RR. The PI-a1-antitrypsin variant PI*WFinnertyon in a family of Caucasian origin. Hum Genet 1985; 69:190-91
17 Tattersall SF, Pereira BP, Hunter D, Blundell C, Pride NB. Lung distensibility and airway function in intermediate alpha1-antitrypsin deficiency (Pi MZ). Thorax 1979; 34:637-46
19 Laros and Klasen (in preparation)