The effect of positive end-expiratory pressure on respiratory resistive properties in anaesthetized paralysed humans

R. Cohendy, J. Ripart, J-J. Eledjam

ABSTRACT: The respiratory resistive properties of the normal human respiratory system are volume-dependent. The overall flow resistance ($R_{\text{max,rs}}$) can be partitioned into airway resistance ($R_{\text{aw}}$) and the additional resistance ($\Delta R_{\text{rs}}$) which may result from the viscoelastic properties of the respiratory system, from inequality of time constants (pendelluft), or from both. Because positive end-expiratory pressure (PEEP) increases end-expiratory lung volume and may equalize ventilation within the lungs, the effect of PEEP on $R_{\text{aw}}$, $\Delta R_{\text{rs}}$, and their sum ($R_{\text{max,rs}}$) was assessed in anaesthetized surgical patients without evidence of lung disease.

Fifteen men were studied during paralysis and isoflow isovolume mechanical ventilation, using the end-inflation occlusion method. Ten men were studied with incremental levels of PEEP, up to 16 cmH₂O (Group A). Five men were studied without PEEP (Group B).

In Group A, $R_{\text{max,rs}}$ did not change with PEEP. In contrast, $R_{\text{aw}}$ decreased and $\Delta R_{\text{rs}}$ increased significantly. Moreover, there was a linear relationship between PEEP and the contribution of $\Delta R_{\text{rs}}$ to $R_{\text{max,rs}}$. In Group B, $R_{\text{max,rs}}$, $R_{\text{aw}}$ and $\Delta R_{\text{rs}}$, and the contribution of $\Delta R_{\text{rs}}$ to $R_{\text{max,rs}}$ did not change. In both groups, atropine elicited a decrease in $R_{\text{max,rs}}$, linked to a decrease in $R_{\text{aw}}$, without any notable effect on the static elastance of the respiratory system ($E_{st,rs}$) or on $\Delta R_{\text{rs}}$.

We conclude that the overall flow resistance was not affected by PEEP. In contrast, PEEP clearly modified the contribution of its two components. The decrease in $R_{\text{aw}}$ with PEEP could have resulted, at least in part, from modification in the basal vagal tone.

operative microscope. The exclusion criteria were: any clinical or radiological abnormality of the respiratory system, smoking (more than 5 packs-yr⁻¹), suspected (history of atopy [6]) or overt (history of wheezes) bronchial hypersensitivity, treatment with a β-blocker. With respect to the experimental protocol, the subjects were divided in two groups: 10 patients were studied with incremental levels of PEEP (Group A), five men were studied without application of external PEEP (Group B). The physical characteristics of the men are shown in the table 1.

Table 1. – Physical characteristics of the two study groups, nature of anaesthetics and doses given for induction of intravenous general anaesthesia

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Age yrs</th>
<th>Height cm</th>
<th>Weight kg</th>
<th>Methohexitone mg·kg⁻¹</th>
<th>Fentanyl µg·kg⁻¹</th>
<th>Vecuronium mg·kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>10</td>
<td>35±2.5</td>
<td>176±5</td>
<td>78±13</td>
<td>3.15±0.39</td>
<td>4.94±0.12</td>
<td>0.20±0.01</td>
</tr>
<tr>
<td>Group B</td>
<td>5</td>
<td>31±5.5</td>
<td>175±4</td>
<td>74±7</td>
<td>3.27±0.63</td>
<td>5.00±0.13</td>
<td>0.18±0.02</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD.

Material

Mechanical ventilation was performed with a Servo Ventilator 900 D (Siemens Elema, Solna, Sweden), which provides PEEP. This ventilator gives a constant inflow (V̇), and an accurate V̇, and allows end-inspiratory occlusion. The initial drop in pressure (Pmax-P1) reflects the resistive pressure attributable to the conducting airway resistance (Raw) during the constant flow preceding the occlusion (V̇) [13, 14]. Thus, Raw equals (Pmax-P1)/V̇. This initial drop in pressure can be underestimated because there is residual gas flowing through the closing valve. This error was minimized by using correction of the closure time of the valve with a linear extrapolation on the paper chart of the pre- and post-occlusion pressure signals to the point in time when the valve was half-closed [15]. Similarly, overall resistance Rmax,rs, was calculated as Rmax,rs = (Pmax-Pel)/V̇; additional resistance (∆Rrs) = Rmax,rs - Raw. In order to take the weight and the height of the subjects into account, the contribution of ∆Rrs to Rmax,rs was calculated as ∆Rrs/Rmax,rs. The tracheal pressure at end-expiration, due to the applied PEEP, was measured by an end-expiratory occlusion performed at the ventilator valves (while pressing the "expiratory hold" button). The static elastance of the respiratory system (Estr,rs) was calculated as (Pel-PEEP)/V̇T [12]. It would have been necessary to take account of any intrinsic PEEP in calculating elastance, but this was never seen in our patients. To avoid absorption atelectasis, the lungs were ventilated with 30% O₂ in nitrogen [16]. At each experimental time, at least one end-expiratory occlusion followed by at least three end-inspiratory occlusions were performed. All of the occlusions were separated by five tidal breaths. As respiratory mechanics are flow and frequency-dependent [3], all the patients underwent mechanical ventilation with the same pattern: frequency = 10 breaths·min⁻¹, VT=0.9 l, TI=1.5 s, V̇=0.6 l·s⁻¹.

Anaesthesia

The supine subjects were studied during intravenous general anaesthesia and paralysis. The study was performed in the operating room, before the beginning of the surgical procedure. Midazolam 5 mg i.m., was given as premedication. Anaesthesia was induced with methohexitone and fentanyl (induction doses, see table 1) and maintained with a continuous infusion of these drugs (methohexitone 0.1 mg·kg⁻¹·min⁻¹; fentanyl 0.1 µg·kg⁻¹·min⁻¹) [17, 18]. Paralysis was produced with vecuronium bromide, and monitored with train-of-four stimulation of the adductor pollicis muscle. A surgical level of paralysis was observed in every subject during the duration of the study. The trachea was intubated with the orotracheal tube described above, the lungs ventilated manually with several large breaths in order to equalize the volume history, and the patient was connected to the ventilator.
Protocol

Group A. Baseline values were recorded 6 min after connection to the ventilator. Four consecutive measurements were performed during the last minute of 4 min steps of incremental levels of PEEP (4, 8, 12 and 16 cmH\textsubscript{2}O) without any other change in the respiratory pattern. To avoid change in the volume history, the lungs were not allowed to return to ventilatory resting level between each step of incremental PEEP. PEEP was released after the 16 cmH\textsubscript{2}O step, and respiratory mechanics parameters were recorded 3 min later. Atropine, 1 mg i.v., was given immediately after the release of PEEP, 3 min before the last measurement.

Group B. The protocol was identical in all aspects to that for Group A, but PEEP was not applied.

Statistical analysis

Data are given as mean±standard deviation. The means were compared with two-way analysis of variance for repeated measures (ANOVA) and paired Student’s t-test. The value of p<0.05 was considered to be significant. When paired t-tests were used to compare the effect of incremental PEEP (i.e. five comparisons) a Bonferroni correction was applied to the critical p-value for significance (p<0.01).

Results

Effect of PEEP

Group A was studied to determine the effect of PEEP. The values measured at baseline, and with incremental levels of PEEP were considered. Est\textsubscript{rs} significantly (ANOVA p=0.034) decreased from baseline as PEEP was set to 4 cmH\textsubscript{2}O (p=0.007) and to 8 cmH\textsubscript{2}O (p=0.01). Est\textsubscript{rs} reached a plateau as PEEP was further increased to 12 cmH\textsubscript{2}O and 16 cmH\textsubscript{2}O. Rmax\textsubscript{rs} remained stable with PEEP (ANOVA p=0.58). By contrast, Raw decreased (ANOVA p=0.0001). Raw with 12 cmH\textsubscript{2}O PEEP (-36%, p=0.0001) and with 16 cmH\textsubscript{2}O PEEP (-48%, p=0.0001) was significantly lower than at baseline. As Rmax\textsubscript{rs} remained stable and Raw decreased, ∆Rrs increased significantly (ANOVA p=0.0021). ∆Rrs was significantly higher than baseline with 12 cmH\textsubscript{2}O PEEP (+10%, p=0.007) and with 16 cmH\textsubscript{2}O PEEP (+23%, p=0.004). The contribution of ∆Rrs to Rmax\textsubscript{rs} (\frac{∆Rrs}{Rmax\textsubscript{rs}}) increased significantly (ANOVA p=0.0001) with the magnitude of PEEP. There was a significant relationship between the level of PEEP and ∆Rrs/Rmax\textsubscript{rs} with the linear regression: \frac{∆Rrs}{Rmax\textsubscript{rs}} (%) = 63.57 + 0.99 \times \text{PEEP} (cmH\textsubscript{2}O).

Table 2. – Effect of PEEP and atropine on respiratory mechanics in Group A (n=10)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>0</th>
<th>Atropine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Est\textsubscript{rs} cmH\textsubscript{2}O·l\textsuperscript{-1}</td>
<td>11.3±1.94</td>
<td>10.6±1.88*</td>
<td>10.3±1.62*</td>
<td>10.5±1.54</td>
<td>10.8±1.27</td>
<td>11.3±1.66</td>
<td>11.7±2.09</td>
</tr>
<tr>
<td>Rmax\textsubscript{rs} cmH\textsubscript{2}O·l\textsuperscript{-1}·s\textsuperscript{-1}</td>
<td>5.6±1.04</td>
<td>5.6±1.07</td>
<td>5.4±0.86</td>
<td>5.2±1.14</td>
<td>5.3±1.11</td>
<td>5.5±0.96</td>
<td>4.4±0.85+</td>
</tr>
<tr>
<td>Raw cmH\textsubscript{2}O·l\textsuperscript{-1}·s\textsuperscript{-1}</td>
<td>2.1±0.70</td>
<td>1.9±0.68</td>
<td>1.7±0.50*</td>
<td>1.4±0.62**</td>
<td>1.1±0.53**</td>
<td>1.8±0.52</td>
<td>1.1±0.33++</td>
</tr>
<tr>
<td>∆Rrs cmH\textsubscript{2}O·l\textsuperscript{-1}·s\textsuperscript{-1}</td>
<td>3.5±0.46</td>
<td>3.7±0.55</td>
<td>3.8±0.65</td>
<td>3.8±0.67*</td>
<td>4.2±0.73*</td>
<td>3.7±0.53</td>
<td>3.2±0.56</td>
</tr>
<tr>
<td>∆Rrs/Rmax\textsubscript{rs} %</td>
<td>62.8±6.40</td>
<td>67.1±6.34*</td>
<td>69.6±6.88*</td>
<td>74.5±7.01**</td>
<td>80.4±6.14**</td>
<td>67.5±5.26</td>
<td>73.3±7.78</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD. Comparison of incremental levels of PEEP to baseline (paired Student’s t-test, Bonferroni correction): *: p<0.01; **: p<0.001. Effect of atropine (versus PEEP 0 cmH\textsubscript{2}O) (paired Student’s t-test): +: p<0.01; ++: p<0.001. PEEP: positive end-expiratory pressure; Est\textsubscript{rs}: static elastance of the respiratory system; Rmax\textsubscript{rs}: overall flow resistance of the respiratory system; Raw: airway resistance; ∆Rrs: additional resistance which may result from viscoelastic properties of the respiratory system from inequality of time constants (pendelluft) or from both.

Table 3. – Effects of time (in minutes elapsed after baseline measurement) and of atropine on the respiratory mechanics of Group B (n=5)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>4th min</th>
<th>8th min</th>
<th>12th min</th>
<th>16th min</th>
<th>20th min</th>
<th>Atropine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Est\textsubscript{rs} cmH\textsubscript{2}O·l\textsuperscript{-1}</td>
<td>12.8±3.52</td>
<td>13.3±3.42</td>
<td>13.5±3.34</td>
<td>13.5±3.45</td>
<td>13.5±3.78</td>
<td>13.9±3.92</td>
<td>14.0±3.48</td>
</tr>
<tr>
<td>Rmax\textsubscript{rs} cmH\textsubscript{2}O·l\textsuperscript{-1}·s\textsuperscript{-1}</td>
<td>6.2±2.17</td>
<td>6.4±2.03</td>
<td>6.3±1.76</td>
<td>6.2±1.92</td>
<td>6.1±1.61</td>
<td>6.1±1.39</td>
<td>4.2±0.63</td>
</tr>
<tr>
<td>Raw cmH\textsubscript{2}O·l\textsuperscript{-1}·s\textsuperscript{-1}</td>
<td>2.8±1.89</td>
<td>2.9±1.61</td>
<td>2.7±1.43</td>
<td>2.8±1.71</td>
<td>2.6±1.22</td>
<td>2.5±1.14</td>
<td>1.2±0.34</td>
</tr>
<tr>
<td>∆Rrs cmH\textsubscript{2}O·l\textsuperscript{-1}·s\textsuperscript{-1}</td>
<td>3.4±0.91</td>
<td>3.3±0.42</td>
<td>3.5±0.83</td>
<td>3.5±0.81</td>
<td>3.5±0.73</td>
<td>3.6±0.54</td>
<td>2.9±0.73</td>
</tr>
<tr>
<td>∆Rrs/Rmax\textsubscript{rs} %</td>
<td>57.4±17.40</td>
<td>55.4±15.66</td>
<td>57.8±14.04</td>
<td>57.8±15.27</td>
<td>59.2±11.43</td>
<td>60.2±11.28</td>
<td>70±9.30</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD. For abbreviations see legend to table 2.
Table 4. – Individual relationships between the contribution of \( R_{rs} \) to \( R_{max,rs} \) (\( \Delta R_{rs}/R_{max,rs} \)) (%) and expiratory pressure (cmH\(_2\)O) assessed by linear regression in Group A (n=10)

<table>
<thead>
<tr>
<th>Pt No.</th>
<th>Intercept</th>
<th>Slope</th>
<th>( r )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>57.58</td>
<td>1.26</td>
<td>0.81</td>
<td>0.09</td>
</tr>
<tr>
<td>2</td>
<td>70.72</td>
<td>0.92</td>
<td>0.96</td>
<td>0.01</td>
</tr>
<tr>
<td>3</td>
<td>57.19</td>
<td>1.64</td>
<td>0.98</td>
<td>0.004</td>
</tr>
<tr>
<td>4</td>
<td>68.07</td>
<td>0.85</td>
<td>0.90</td>
<td>0.03</td>
</tr>
<tr>
<td>5</td>
<td>52.53</td>
<td>1.04</td>
<td>0.96</td>
<td>0.01</td>
</tr>
<tr>
<td>6</td>
<td>69.41</td>
<td>0.64</td>
<td>0.92</td>
<td>0.02</td>
</tr>
<tr>
<td>7</td>
<td>64.9</td>
<td>1.31</td>
<td>0.89</td>
<td>0.04</td>
</tr>
<tr>
<td>8</td>
<td>63.12</td>
<td>1.42</td>
<td>0.90</td>
<td>0.03</td>
</tr>
<tr>
<td>9</td>
<td>65.31</td>
<td>0.45</td>
<td>0.64</td>
<td>0.24</td>
</tr>
<tr>
<td>10</td>
<td>64.96</td>
<td>1.09</td>
<td>0.86</td>
<td>0.06</td>
</tr>
</tbody>
</table>

For abbreviations see legend to table 2.

\( r \leq 0.63, p=0.0001 \), with a difference between the mean observed value of \( \Delta R_{rs}/R_{max} \) and its predicted value at the intercept of -0.79. The individual relationships of \( \Delta R_{rs}/R_{max,rs} \) and PEEP are summarized in table 4. Among the 10 subjects studied, seven (patients Nos 2–8) showed a significant and close relationship.

**Effect of the release of PEEP**

Group A was studied to determine the effect of release of PEEP (table 2). \( R_{st,rs} \) returned to its baseline value when PEEP was released (baseline 11.25±1.94 cmH\(_2\)O; PEEP 0 11.27±1.66 cmH\(_2\)O). \( R_{max,rs} \), \( R_{raw} \), \( \Delta R_{rs} \) and \( \Delta R_{rs}/R_{max,rs} \) were very close to their baseline values when PEEP was released.

**Effect of time**

Group B was studied to determine the effect of time (table 3). All experimental times, except “atropine” time, were considered. \( R_{st,rs} \), \( R_{max,rs} \), \( R_{raw} \), \( \Delta R_{rs} \) and \( \Delta R_{rs}/R_{max,rs} \) remained stable.

**Effect of atropine**

The parameters recorded when PEEP was released (Group A, table 2) and at the 20th minute for Group B were compared to the parameters measured under atropine in each group. These parameters were not pooled. The mean values of the \( R_{st,rs} \) after the administration of atropine were very close to their values when PEEP was released in Group A and at the 20th minute in Group B. The decrease in \( R_{max,rs} \) with atropine was significant in the two groups: -20% in Group A (\( p=0.01 \)), -31% in Group B (\( p=0.015 \)). \( R_{raw} \) decreased significantly in Group A: -40%, (\( p=0.001 \)), but the decrease in \( R_{raw} \) in Group B (-51%) did not reach statistical significance (\( p=0.07 \)).

**Discussion**

PEEP elicited significant changes in the respiratory mechanics of anaesthetized, surgical patients with normal lungs. \( R_{st,rs} \) decreased up to a PEEP of 8 cmH\(_2\)O. Since \( R_{max,rs} \) remained stable with PEEP, PEEP clearly modified the contribution of \( R_{raw} \) and of \( \Delta R_{rs} \), as \( R_{raw} \) decreased. Therefore, the contribution of \( \Delta R_{rs} \) to \( R_{max,rs} \) increased with PEEP, up to 80% of the value of \( R_{max,rs} \). Moreover, as PEEP was released, respiratory mechanics returned to baseline, and the duration of the study was not a confounding factor. Finally, atropine induced a substantial bronchodilation, without notable effect on \( R_{st,rs} \).

Intravenous general anaesthesia avoided the bronchodilatory effect of halogenated anaesthetics, and their effect on pulmonary tissue resistance [19]. If the opioid fentanyl increases \( R_{max,rs} \), and induces a parallel increase in both \( R_{raw} \) and \( \Delta R_{rs} \) when it is given as a 5 \( \mu \)g·kg\(^{-1}\) bolus during barbiturate general anaesthesia [10], its effect on the pulmonary resistance is stable when it is given as a continuous infusion [20]. As the continuous infusion of anaesthetics gives a predictable plasma concentration [17, 18], a stable effect on resistances was expected and was confirmed by the control study of Group B.

The EIOM allows \( R_{max,rs} \) to be partitioned into \( R_{raw} \) and \( \Delta R_{rs} \) [2]. As respiratory mechanics vary with the ventilatory pattern [3], we maintained the same pattern for the sake of comparison to the baseline resistance of Group A, we have derived from the data reported by this group the predicted values of resistance with the inspiratory
volume (0.9 l) applied to our subjects. The inflation flow that we used (0.6 l s⁻¹) was close to the mean flow used by D'ANGELO and co-workers [3] (0.557 l s⁻¹). Rmax.rs was estimated to be 5.53 cmH₂O l⁻¹ s⁻¹ as compared to the measured baseline value of 5.57 cmH₂O l⁻¹ s⁻¹ (table 2). Similarly, Raw was predicted at 1.89 cmH₂O l⁻¹ s⁻¹ and was measured at 2.11 cmH₂O l⁻¹ s⁻¹, and ∆Rrs was predicted as 3.63 cmH₂O l⁻¹ s⁻¹ and was measured at 3.45 cmH₂O l⁻¹ s⁻¹. ∆Rrs/Rmax.rs was estimated to be 66%, as compared to the baseline value in our study (63%): the large contribution of ∆Rrs to Rmax.rs was probably due to the high V₁. Therefore, the baseline resistances measured in our study were close to those reported by D'ANGELO and co-workers [3], who had to correct Raw for the resistive pressure drop due to the endotracheal tube. Moreover, in vitro measurement may not accurately predict the effect of the tube in vivo [22]. In our study, the airway pressure was measured at the tracheal tip of the endotracheal tube, in order to avoid this subtraction [11]. The Bernoulli effect could have affected the measurement of pressure, leading to underestimation of actual tracheal pressure. However, the magnitude of this effect varies with the flow; because the inspiratory flow was the same in each case, the error resulting from this effect was not a confounding factor. The close similarity of our values with those derived from the work of D'ANGELO and co-workers [3] suggests that the procedure we had chosen, although simplified, was valid.

Flow resistances returned to control values when PEEP was released in Group A. In Group B there were no sizeable variations in resistance with time or with unexpected variation in the depth of the anaesthesia. Therefore, the variations in the flow resistances observed in our study can be assigned to the effect of incremental PEEP.

Rmax.rs was insensitive to the effect of PEEP. This has also been described in anaesthetized and mechanically-ventilated normal subjects by PESENTI et al. [23], who measured this resistance for PEEP up to 10 cmH₂O. In our study, Rmax.rs decreased with atropine in both groups, mainly because of a consistent decrease in Raw, which demonstrated the presence of a vagal tone at baseline. On the other hand, Raw decreased with PEEP, up to 48% of its baseline value at 16 cmH₂O. The significant decrease in Est.rs with lower levels of PEEP (up to 8 cmH₂O) (table 2) was consistent with opening of lung units; the decrease in Raw should have been related to effective increase in lung volume. Indeed, it is generally held that Raw is inversely related to lung volume [4]. By contrast, some overdistension (signalled by increase in Est.rs) occurred with higher levels of PEEP, but Raw still decreased almost linearly. Thus, the bronchodilating effect of PEEP may not be purely mechanical in nature. Indeed, it has been suggested that PEEP could have a vagally-mediated inhibitory influence, resulting from the stimulation of pulmonary stretch receptors [24]. It should be noted that the initial pressure drop after occlusion has been shown to reflect Raw, despite introduction of mechanical heterogeneities [25].

As Raw decreased, ∆Rrs/Rmax.rs increased, up to 80% when PEEP was set to 16 cmH₂O, as compared to 62% at baseline (table 2). ∆Rrs may result from the peripheral additional impedance caused by stress relaxation of the respiratory tissues (viscoelasticity) and/or time constant inhomogeneities between lung units (pendelluft) [3, 14, 26, 27]. The pendelluft phenomenon is generally held as negligible in normal conscious subjects [1]. However, it has been shown in surgical patients [28] that at the very beginning of a general anaesthesia with paralysis, dependent area of consolidation develop within the lung. These so-called compression atelectatic areas are no longer detectable when PEEP is applied [29]. Because in our patients Est.rs decreased with the lower levels of PEEP, suggesting opening of closed lung units, ∆Rrs at baseline could have resulted, at least in part, from heterogeneities related to such atelectases. Nevertheless, if baseline ∆Rrs actually reflected a substantial pendelluft phenomenon, it should have decreased (and not increased) with PEEP in our patients. Moreover, the viscoelastic model has been shown to be the most appropriate two compartment model for describing normal canine pulmonary mechanics with EiOM [13] within the tidal volume range. PESENTI et al. [23], in a study that did not specifically address the variations of ∆Rrs or of ∆Rrs/Rmax.rs with PEEP, reported a nonsignificant trend of ∆Rrs to increase with PEEP (up to 10 cmH₂O) in normal subjects. More recently, D'ANGELO and co-workers [26] found that external PEEP (7.76±1.83 cmH₂O) during mechanical ventilation did not change ∆Rrs. This is in line with the present results, because ∆Rrs increased significantly only with a PEEP of 12 cmH₂O (table 2). Therefore, we speculate that pendelluft had only a small role, if any, in the creation of ∆Rrs at baseline in our patients. The increase in ∆Rrs with the higher levels of PEEP should have signalled the development of mechanical inhomogeneities within the respiratory system.

In conclusion, in surgical patients without evidence of respiratory disease, PEEP did not affect the overall flow resistance of the respiratory system, but clearly modified the distribution of ∆Rrs and Raw. The decrease in Raw with PEEP could have resulted, at least in part, from modification of the basal vagal tone. It seemed that the pendelluft phenomenon had a only small role, if any, in the creation of ∆Rrs at baseline.

References


