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# Pulmonary intravascular volume can be used for dose calculation in isolated lung perfusion

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**Abstract**

**Introduction**: Isolated lung perfusion (ILuP) is an experimental surgical technique for the treatment of pulmonary metastases. Phase I trials showed a wide range in drug lung levels. This may be due to the variance of lung size and pulmonary intravascular volume (PIV). Therefore, we developed a method to assess PIV and investigated the relation of PIV and dry lung weight (DLW). **Material and methods**: Thirty-two rats of 555±8 and 199±5 g underwent left ILuP two, four and eight minutes. Venous effluent was analyzed for haemoglobin, red blood cells (RBC), leucocytes, platelets, albumin and creatinine. PIV was calculated by dividing the product of perfusate volume and post-ILuP parameter by the difference between post-ILuP and pre-ILuP parameter. **Results**: No significant differences in PIV for all perfusion times were noted between the different variables ($P=0.14$). Based on haemoglobin ($P<0.0009$), RBC ($P=0.006$), leucocytes ($P=0.0003$), platelets ($P=0.017$) and creatinine ($P=0.003$) analysis, PIV was significantly smaller in rats of 199 g while PIV/DLW ratio was not significantly different. **Conclusion**: Because PIV/DLW ratio is independent of body weight, we advocate PIV calculation using haemoglobin and RBC as an excellent parameter for drug dose calculation during ILuP intraoperatively in order to achieve more reproducible local drug levels and higher efficacy.


*Keywords:* Pulmonary intravascular volume; Isolated lung perfusion; Experimental; Rat model

## 1. Introduction

The number of patients suffering from colorectal cancer expected to die in the USA for 2004 has been estimated at 563,700 [1]. A significant amount of these patients will develop lung metastases. Without any treatment, patients with pulmonary metastatic disease have no 5-year survival. However, surgical resection of metastatic nodules results in 40% 5-year survival while intravenous administration of chemotherapeutics has no additive effect due to dose-limiting toxicity [2]. Most patients die because of locoregional recurrences, probably originating from micrometastases already present at the initial surgery.

Isolated lung perfusion (ILuP) with cytostatic drugs is an experimental surgical technique in order to improve prognosis of these patients. Significantly higher local tissue drug levels are achieved due to the lack of systemic toxicity resulting in higher tumour cell kill [3].

Several drugs like melphalan, gemcitabine, doxorubicin and cisplatin have successfully been tested in animal models resulting in elongation of survival and even complete remission [4–7]. For example, combinations of gemcitabine, melphalan and cisplatin were tested either in vitro or in vivo in our laboratory. ILuP monotherapy with these drugs resulted in significantly longer survival compared to untreated controls while synergistic actions were observed after combination therapy with gemcitabine with melpalan and cisplatin with melphalan [8].

The experimental work resulted in several phase I trials [9–13]. Dose-escalating schedules were applied searching for acceptable toxicity levels. Obviously, these human studies showed a wide range of final lung and tumour levels resulting in subtherapeutical concentrations in a significant number of patients [11–13].

We hypothesize that one major reason for this variance in final local drug levels is based on inter human differences in dry lung weight and therefore in pulmonary intravascular volume (PIV) leading to an important variability in initial perfusate levels. Therefore, we aimed to create a method to assess PIV intraoperatively and second to investigate the relation of PIV and dry lung weight.

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## 2. Material and methods

### 2.1. Animals

Male inbred Wistar rats (weight: 199±5 and 555±8 g), obtained from Iffa Credo (Brussels, Belgium), were used for all experiments. Animals were treated in accordance with the Animal Welfare Act and the 'Guide for the Care and Use of Laboratory Animals' (NIH Publication 86-23, revised 1985). The rats were transported in sterile conditions, housed in suspended mesh wired cages and fed a standard pellet diet ad libitum (standard rat chow, Hope Farms, Woerden, The Netherlands). The experimental protocols were approved by the Institutional Animal Care and Use Committee, University Hospital of Antwerp.

### 2.2. Technique of left isolated lung perfusion

The technique of ILuP has extensively been described in earlier reports [14]. Briefly, anesthesia was induced with halothane during three minutes. Rats were incubated by translaryngeal illumination and connected to the ventilator. Halothane was titrated between 0.5 and 1.5% according to muscle relaxation, heart rate and pupil size. Ventilation was accomplished with a volume-controlled ventilator at a rate of 75 strokes/min and a tidal volume of 10 ml/kg. After a left thoracotomy was performed, the lung and rib retractor were placed anteriorly. The hilum was dissected free under microscopic view. The bronchus was deprived from its surrounding tissue in order to exclude the bronchial arterial flow to the lung. Two 16-G angiocatheters were placed through the chest wall to facilitate cannulation of the pulmonary vessels. The pulmonary artery and common trunk vein were proximally clamped with curved microclips simultaneously, before another two clamps were placed distally on both the pulmonary artery and the inferior and superior pulmonary veins. Then, two PE-10 perfusion catheters were introduced into the chest through both angiocatheters. The pulmonary artery and common trunk vein were cannulated between two clamps without blood loss while the cannulas were secured by a 4/0 silk tie after insertion. After removing the distally placed clamps, the venous cannula, which was not flushed, lies in the common trunk vein in order to discard venous effluent coming from both the inferior and superior pulmonary vein. Effluent was collected in a 500 μl $K_2$-EDTA Microtainer (BD, Franklin Lakes, NJ, USA). Perfusate was delivered with a flow rate of 0.2 ml/min through the flushed arterial catheter and buffered starch (Haes steril 6%, Fresenius Kabi, Schelle, Belgium) was used for all perfusions. Perfusate temperature was controlled at 37 °C throughout the whole perfusion. Rats were placed on a heating pad immediately after induction, and body temperature was kept constantly between 36 and 37 °C.

### 2.3. Analysis of venous effluent

All fluid determinations were processed within 4 h after sampling. Hematological screening (haemoglobin (Hb), white blood cells (WBC), platelets (PLT) and red blood cells RBC)) was performed using a Cell-Dyn 4000 hemocytometer (Abbott Laboratories, Santa Clara, CA, USA) while chemical analyses (albumin, Cr and total protein) were performed using a Vitros 950 apparatus (Ortho Clinical Diagnostics, Rochester, NY, USA).

### 2.4. Statistical analysis

All results are reported as mean±standard error. ANOVA analysis was applied to compare PIV between different perfusion times for each hematological and chemical parameter while a separate analysis was performed to compare PIV from the different perfusion times between the different hematological and chemical variables. Student's *t*-test was used to compare wet and dry lung weight, wet-to-dry ratio, PIV and PIV/dry lung weight between rats of 199±5 and 555±8 g.

Outliers were detected with Grubb's test. Statistical significance was accepted at $P<0.05$.

### 2.5. Experiment

In order to determine the optimal perfusion time for PIV calculation, 25 rats (mean 555±8 g) (groups 1–3) were randomized into three groups. Groups 1 ($n=9$), 2 ($n=9$) and 3 ($n=7$) underwent left ILuP during 2, 4 and 8 min, respectively. PIV was calculated by:

$$\mathrm{PIV} = (V_p \times X_{\mathrm{end-ILuP}})/(X_{\mathrm{initial-ILuP}} - X_{\mathrm{end-ILuP}})$$

| | |
|---|---|
| PIV | pulmonary intravascular volume |
| ILuP | isolated lung perfusion |
| $X_{\mathrm{end\text{-}ILuP}}$ | analyzed haematological or chemical value in the perfusate after ILuP |
| $X_{\mathrm{initial\text{-}ILuP}}$ | analyzed haematological or chemical value in rat blood before ILuP |
| $V_p$ | perfusate volume (perfusion time×0.2 ml/min) |

In order to prove that the ratio PIV/dry lung weight is constant implying that PIV can be used for drug dose calculation during ILuP intraoperatively, another group of rats (mean 199±5 g) (group 4, $n=7$) was added. Because the proposed method of PIV calculation is adequate for all perfusion times within the studied flow and perfusion time range, 4 min of left ILuP under identical study conditions was chosen ad random for group 4.

## 3. Results

Two rats of 34 were excluded from this study because of blood leakage during cannulation.

The analyzed 'end-ILuP' samples contained levels below the detection limit of total protein in 20% and albumin in 12%. These missing values were replaced by the lowest measurable concentration (10 g/l for total protein and 4 g/l for albumin).

Fig. 1 shows dilution graphs of analyzed hematological and chemical variables in function of time while calculated PIV is depicted in Fig. 2 in function of time.

No significant differences in PIV were observed between different perfusion times for Hb, RBC, WBC, PLT and Cr while significant differences were shown for

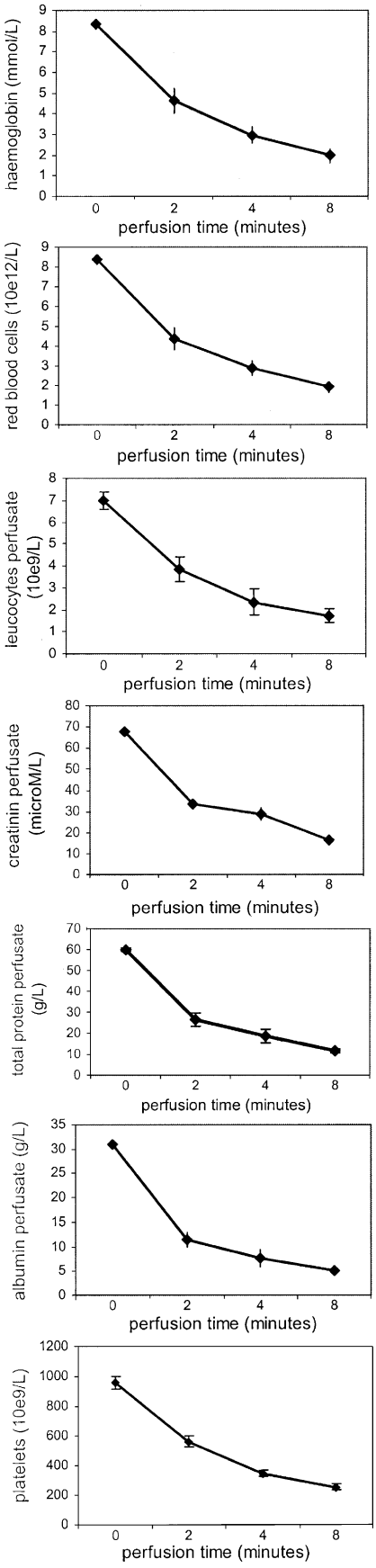


Fig. 1. Dilution graphs of analyzed haematological and chemical variables in function of time.

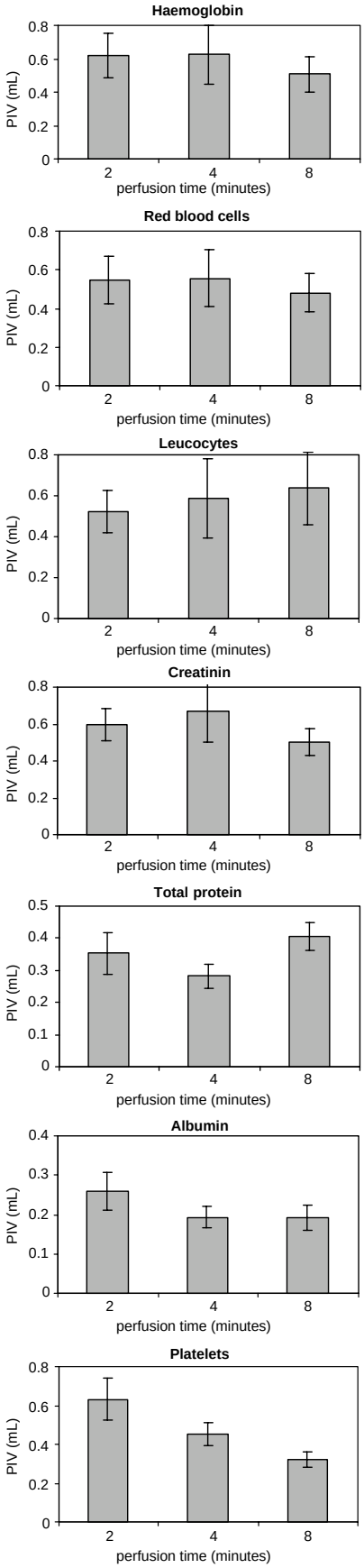


Fig. 2. Calculated PIV values in function of time depicted as mean ± standard error.

Table 1
PIV and PIV/dry lung weight calculated with Hb, RBC, WBC, PLT and Cr and compared between rats of 555 and 199 g

| | Wet | Dry | W/D | PIV (Hb) | PIV/dry (Hb) | PIV (RBC) | PIV/dry (RBC) | PIV (Cr) | PIV/dry (Cr) | PIV (WBC) | PIV/dry (WBC) | PIV (PLT) | PIV/dry (PLT) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *555 g* | | | | | | | | | | | | | |
| Mean | 1.05 | 0.18 | 5.9 | 0.56 | 2.65 | 0.51 | 2.22 | 0.53 | 2.80 | 0.57 | 3.03 | 0.56 | 2.76 |
| SE | 0.21 | 0.036 | 1.2 | 0.078 | 0.32 | 0.070 | 0.22 | 0.050 | 0.35 | 0.09 | 0.51 | 0.11 | 0.44 |
| *199 g* | | | | | | | | | | | | | |
| Mean | 0.83 | 0.13 | 6.4 | 0.19 | 1.50 | 0.27 | 2.01 | 0.32 | 2.09 | 0.20 | 1.60 | 0.16 | 1.23 |
| SE | 0.17 | 0.026 | 1.3 | 0.041 | 0.33 | 0.034 | 0.24 | 0.028 | 0.10 | 0.020 | 0.51 | 0.048 | 0.39 |
| *P*-value | 0.0030 | 2.76E-05 | NS | 0.00094 | NS | 0.0060 | NS | 0.0032 | NS | 0.00031 | 0.017 | 0.012 | NS |

PIV, pulmonary intravascular volume; W/D, wet-to-dry ratio; Hb, haemoglobin; RBC, red blood cells; WBC, white blood cells; PLT, platelets; Cr, creatinine; Wet, wet lung weight; Dry, dry lung weight; SE, standard error.

albumin ($P=0.014$) and total protein ($P=0.022$). No significant differences in PIV for all perfusion times were shown between the different hematological and chemical variables ($P=0.14$).

Wet ($P=0.003$) and dry ($P=0.00003$) lung weight was significantly lower in rats of 199 g while wet-to-dry ratio did not differ significantly. PIV calculated for rats of 199 g was significantly lower compared to animals of 555 g using Hb ($P=0.0009$), RBC ($P=0.006$), Cr ($P=0.003$), PLT ($P=0.012$) and WBC ($P=0.0003$). Calculation of PIV/dry lung weight did not result in significant differences for Hb, RBC, Cr and PLT while PIV/dry lung weight was significantly higher in rats of 555 g ($P=0.02$) using WBC (Table 1).

## 4. Discussion

To our knowledge, this is the first study that developed a method to calculate PIV intraoperatively. We furthermore showed a constant relation between PIV and dry lung weight being independent of body weight.

No significant differences in PIV were observed using hematological blood values and Cr in function of perfusion time. However, PIV was not constant using albumin and total protein (Fig. 2). A large number of the analyzed 'end-ILuP' samples contained levels below the limit of detection of total protein (20%) and albumin (12%) resulting in inaccurate PIV values. Therefore, these two variables were excluded from PIV/dry lung weight calculation.

ANOVA analysis showed no significant differences in PIV between the variables used. From these results, it can be concluded that PIV can be calculated using Hb, RBC, WBC, PLT and Cr analysis independent of perfusion time within the studied range. From these parameters, Hb and RBC are most adequate for PIV calculation intraoperatively.

Hb and RBC analysis is even applicable in the operating theatre. Both analyses are performed in whole blood samples without being centrifuged and therefore are instantly available. In contrast, Cr samples are analyzed after being centrifuged which is time-consuming. However, renal function and muscle mass are expected to be stable during ILuP resulting in comparable accuracy of creatinine for PIV calculation compared to Hb and RBC.

Although WBC and PLT proved to be successful parameters for PIV calculation in this experiment, it has to be emphasized that WBC have well known acute phase characteristics while PLT are activated by tubing during ILuP. If leucocyte activation occurs, sequestration, followed by low flow perfusion in significant portions of the lung, will result in underestimation of the number of leucocytes in the perfusate and therefore in underestimation of PIV. On the other hand, PLT activation may result in enhanced clotting activity during inadequate heparinization also leading to hypoperfusion and therefore underestimation of PIV.

We hypothesized that knowledge of dry lung weight is essential for drug dose determination. Because no parameters are known related to dry lung weight, we investigated the relation between dry lung weight and PIV. Using Hb, RBC, WBC, PLT and Cr, significantly lower PIV values were calculated in rats of 199 g compared to rats of 555 g while no significant differences in PIV/dry lung weight were observed for these parameters except for WBC. As mentioned before, WBC are inadequate for PIV calculation and therefore for PIV/dry lung weight determination. This finding confirms our hypothesis that PIV is an easy and cheap method to calculate the drug dose necessary for achieving reproducible initial perfusate drug levels.

It is important to realize that some specific pathological circumstances like lung emphysema might interfere with the PIV/dry lung weight relation. However, a significant part of patients with solitary pulmonary metastases, target patients for treatment with ILuP, suffer from soft tissue sarcomas, osteosarcomas and testicular carcinoma. In contrast to patients suffering from primary bronchogenic carcinoma, this patient group is not characterized by heavy smoking, which is the major cause of lung emphysema.

The experimental results from this study are being validated in humans (unpublished results). Currently, a phase I trial evaluating melphalan in ILuP has recently been completed in our cardiothoracic institutions determining the maximal tolerated dose of melphalan to be 45 mg at 42 °C in the setting of ILuP in humans [9]. Dose limiting toxicity occurred at 60 mg melphalan at 42 °C. After cannulation of the pulmonary artery and veins, several minutes of perfusion without drugs are necessary to get a stable perfusion pressure. During this time interval, one sample for PIV calculation is taken from the circuit after 1 min. With knowledge of PIV and tubing volume, the correct drug dose is calculated in order to achieve reproducible perfusate and local drug levels resulting in augmentation of efficacy of ILuP.

In conclusion, this is the first study that developed a method to calculate PIV intraoperatively in order to achieve a constant initial drug concentration in the perfusion circuit.

We furthermore showed a constant relation between PIV and dry lung weight being independent of body weight. This finding implies PIV to be an independent variable for drug dose calculation in ILuP.

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