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DMD Fast Forward. Published on January 10, 2008 as doi:10.1124/dmd.106.014639

Pharmacokinetics of Gemcitabine when delivered by Selective Pulmonary Artery Perfusion for the Treatment of Lung Cancer

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Running title: selective pulmonary artery perfusion

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Number of text pages: 25

Number of tables: 0

Number of figures: 14

Number of references:

Abstract word count: 250

Introduction word count: 643

Discussion word count: 1734

Non-standard abbreviations: SPAP : selective pulmonary artery perfusion

IV: intravenous

NSCLC: non small cell lung cancer

AUC: area under the curve

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Abstract

Lung cancer represents a major health problem. Cytostatic and radio-therapeutic treatment are both limited due to dose-limiting systemic toxicity and surgery due to its invasive nature. Therefore, we developed a catheterisation model of selective pulmonary artery perfusion (SPAP) combining the properties of isolated lung perfusion and intravenous treatment to achieve higher local drug levels and equivalent systemic exposure. Sixteen pigs underwent SPAP using a clinically applied dose of gemcitabine (1g/m^2). They furthermore underwent thoracotomy for tissue sampling. Three groups were treated with SPAP for two minutes with normal pulmonary blood flow, 50% and 90% flow reduction. Another group had SPAP for ten minutes with normal blood flow. All SPAP groups underwent catheterisation of the left pulmonary artery. An additional group (n=4) was infused intravenously (IV) for thirty minutes using the same dose. Concentrations were analysed with ANOVA. Pulmonary peak concentrations (p=0.01) and areas under the curve (AUC) (p=0.001) of SPAP for two and ten minutes were significantly higher compared to IV while SPAP for ten minutes resulted in the highest AUC (p=0.045) compared to SPAP for two minutes. Flow reduction during SPAP resulted in inhomogeneous distribution. Liver levels, AUC (serum) and wet-to-dry ratios of all SPAP groups were not significantly different compared to IV. SPAP resulted in higher lung concentrations while systemic exposure was comparable with IV. Therefore, we advocate SPAP as a new method to be tested clinically to achieve down-staging of the tumour (T) and lymph node (N) status in lung cancer.

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Introduction

Cancer is the leading cause of death before the age of 85 years resulting in more than half a million deaths per year in the United States (Jemel et al., 2006). In 2005, primary lung cancer was the second leading cancer type in the United States with approximately 190,000 new cases to be estimated for 2006. Among all cancer types, lung cancer has the highest death rate (Jemel et al., 2006).

Non-small cell lung (NSCLC) cancer is usually treated by surgical resection, radiotherapy and/or cytostatic drug administration depending on the disease stage. Stage 1 (a and b) and 2 (a and b) NSCLC are currently treated by surgical resection while (adjuvant) cytostatic therapy is applied to stage Ib, II and III disease resulting in a 5-year survival of 75, 60, 40, 20 and 15% respectively (Spiro et al., 2005).

Intravenous infusion is the desired route of cytostatic drug administration in order to achieve exposure of the primary lung tumour and distant disease as well resulting in a 5-year survival benefit of 4-14% compared to surgery alone (Betticher, 2005). However, this method is dose-limited by the occurrence of systemic toxicity like bone-marrow suppression that limits exposure of the primary tumour and pulmonary (lymph node) metastases.

In contrast, isolated lung perfusion with cytostatic drugs is an experimental surgical technique for the treatment of lung metastases that aims to destroy pulmonary (lymph node) micrometastatic disease probably present at the moment of surgery. This technique is characterized by some properties that could improve current treatment of NSCLC. First, isolated lung perfusion results in significantly higher drug concentrations in both lung and tumour tissue compared to intravenous administration as shown by many experimental and human data (Hendriks et al., 1998 and 2004, Van Putte et al., 2002, Pass et al., 1996, Burt et al. 2000, Ratto et al., 1996). High dose drug administration during isolated lung perfusion using drugs like gemcitabine and cisplatin is well tolerated by healthy lung tissue (Ratto et al.,

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1996, Van Putte et al., 2003[a] and 2005). Furthermore, a recent phase 1 trial evaluating toxicity of isolated lung perfusion with melphalan showed a rapid pulmonary lymph drainage resulting in equivalent concentrations in either the lymph nodes and lung tissue while this approach is applied only once or twice per patient due to its invasive nature [unpublished data]. However, isolated lung perfusion is a local regional treatment modality developed for the treatment of lung metastases, but not for the treatment of NSCLC.

Twenty-six to thirty-two percent of all recurrences are local in patients with stage 1 and 2 NSCLC treated by lobectomy (Rivera et al., 2001). Recurrent disease probably originates from micrometastases present at the moment of surgical resection. In fact, local control is not achieved in these patients after initial surgical and cytostatic treatment. Therefore, this study aimed to develop a hybrid model of selective pulmonary artery perfusion combining the properties of isolated lung perfusion and intravenous treatment in order to improve outcome of NSCLC with minimal side-effects and to achieve down-staging of stage III a and b NSCLC towards a surgical stage.

First, higher drug exposure of the diseased lobe is necessary in order to achieve tumour (T status) size reduction. Second, the residual lung lobes probably contain micrometastases, that have to be treated more aggressively in order to prevent pulmonary recurrences. Third, lymph node status (N status) reduction is essential to achieve down-staging of stage III a and b NSCLC. These lymph nodes that are the first station for metastases after the lung lobes themselves, will be co-treated using high drug levels. Finally, selective pulmonary artery perfusion aims to attack distant disease outside the lungs (M status) in an equivalent way as the currently applied intravenous administration does.

More specifically, in this study, infusion variables of selective pulmonary artery perfusion are optimalized and compared to the standard intravenous route of drug administration.

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Material and Methods

Animals

Twenty female Dutch Landrace pigs (mean weight: 60 ± 3.7 kg) were used. Animals were fed with a normal diet and 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 experimental protocol was approved by the animal experimentation committee of the Utrecht University (04/220).

Anaesthesia and Euthanasia

Anaesthesia was induced with ketamine (10 mg/kg), midazolam (0.5 mg/kg) and atropine (0.04 mg/kg) intramuscularly. Each pig received thiopental natrium 4 mg/kg through an intravenous line. After intubation, the animals were connected to a volume-controlled ventilator (8 mL/kg, 12 breaths/minute guided by capnography) maintaining positive end-expiratory pressure of 5cm of H_20 and an inspiratory oxygen fraction of 0.5. Anaesthesia was maintained by continuous infusion of midazolam (0.7 mg/kg·h). Analgesia was obtained with continuous infusion of sufentanil citrate (10 μg/kg·h) and muscle relaxation with pancuronium (0.1 mg/kg·h). Furthermore, a continuous infusion of saline (300 mL/h) was administered during the operation. After finishing the experiment, animals were sacrificed with pentobarbital natrium (200 mg/kg) intravenously.

A central venous line was inserted for serum sampling during the experiment and a catheter was introduced into the right femoral artery for arterial blood pressure monitoring.

Surgery

Initially, a balloon catheter (Balloon Wedge Pressure Catheter, 7 French, 110 cm, Arrow International, USA) was introduced through the left internal jugular vein. The catheter was

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positioned into the left pulmonary artery under blood pressure guidance. Subsequently, a left sided anterolateral thoracotomy was performed through the fifth intercostal space. The left pulmonary artery was dissected free and the position of the balloon catheter was checked for manually. The tip of the catheter was positioned in the left main stem pulmonary artery just proximal of the first side-branches (fig. 1b).

A 12 mm flow probe was placed around the left pulmonary artery for blood flow measurements just distal of the tip of the balloon catheter and just proximal of the first side branches. In addition, these measurements were necessary to check reduction of the pulmonary artery flow (0%, 50% and 90% blood flow in mL/min reduction). This flow reduction was realized by insufflating the balloon of the balloon catheter.

After stabilisation of the blood flow, gemcitabine was infused through the lumen of the balloon catheter (SPAP) into the left pulmonary artery, or through the central venous line (intravenous administration) using an infusion pump. Tissue samples of the lung were obtained from the left lower lobe and stored in chloroform calcium and liquid N_2 for later analysis. Furthermore, serum samples were collected from the central venous line and stored in tubes filled with 500 µl K_2-EDTA to prevent clotting and immediately frozen into liquid nitrogen. At the end of the experiments, liver samples were obtained through an opening in the right hemi-diaphragm.

Histology

After fixation in chloroform calcium during 90 minutes at room temperature lung tissue was stored in a buffer (10 mL distilled H_2O, 1 g $CaCl_2$, 0.121M Cacodylate) at 4ºC until further processing. Tissue samples for light microscopic investigations were dehydrated with isopropanolol, cleared with toluol and embedded in paraffin wax. 4 µm Sections were stained with haematoxylin and eosin for later assessment.

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Gemcitabine

Gemcitabine (difluorodeoxycytidine, dFdC, Ely Lilly, Indianapolis, USA) solutions were prepared by reconstituting non-lyophilized powder in saline solution. All animals were treated with gemcitabine in a dose and volume (1000 mg/m^2 body surface area, solved in 50 mL saline) as clinically applied for the treatment of NSCLC.

Gemcitabine processing and measurement

A high-performance liquid chromatographic method has been used and validated for the determination of gemcitabine (dFdC) in plasma, lung and liver tissue. Before analysis Tissue and serum samples Standard samples of blanc plasma were spiked with gemcitabine (100 ng-100.000 ng) and extracted in the same way as the other samples and used for a calibration curve (De Boeck et al., 1997). Within-run and between-run precisions were less than 10 % and average accuracies were between 90 and 110 %. Before analysis, the frozen tissue and serum samples are mixed with tetrahydrouridine in order to prevent metabolization by cytidine deaminase.

Gemcitabine assay by HPLC-UV

Separation was achieved on a Chrompack Spherisorb ODS-2 reversed phase column (25 xm x 4.6 mm, 5 µm). The mobile phase used was Pic B7 reagent (Waters Corporation) in 15 % methanol (pH = 3.1) with a flow rate of 1.0 ml/min. Gemcitabine is detected by UV detection at 270 nm.

Statistics

All concentrations and wet-to-dry ratios shown is this paper are depicted as median ± standard error. Lung and serum concentrations are determined in function of time and calculated as

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areas under the curve (AUC). The AUC values and the median concentrations at each single time point were compared between the different groups using ANOVA analysis followed by comparison between two individual groups using Student's t-test. Statistical significance was accepted at $p < 0.05$.

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Experiment

Twenty pigs were randomized into five groups (n=4, each) (fig. 1a). All groups received gemcitabine in a dose of 1 g/m^2, solved in a volume of 50 mL saline.

In order to determine the optimal infusion time for SPAP, two groups underwent SPAP with a normal pulmonary artery blood flow for ten minutes and for two minutes resulting in six and thirty times higher drug concentrations delivered at the tip of the catheter respectively compared to intravenous infusion. A control group was treated intravenously according to a clinically applied regime for the treatment of NSCLC and gemcitabine was infused over a period of 30 minutes.

Two more groups underwent SPAP for two minutes with 50% and 90% flow reduction within the pulmonary artery as checked with the flow probe around the pulmonary artery resulting in sixty and three hundred times higher drug levels delivered at the tip of the catheter respectively compared to intravenous infusion.

After SPAP for 2 minutes at normal or reduced flow rate, the balloon was desufflated and a normal blood flow within the pulmonary artery was maintained throughout the further duration of the experiment.

Lung and serum samples were obtained at two, ten, thirty and fourty-five minutes after start of infusion. Liver samples and lung tissue were taken at fourty-five minutes for concentration analysis, wet-to-dry ratio measurements and histology respectively.

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Results

In lung tissue, SPAP for two and ten minutes resulted in significantly higher AUC when compared to intravenous infusion (p = 0.001) and SPAP for ten minutes resulted in the highest lung AUC compared to SPAP for two minutes (p = 0.045) (fig 2b). In addition, the peak concentration of gemcitabine within the lung tissue was significantly higher after SPAP for two minutes when compared to intravenous administration (p= 0.01) (fig. 2a).
Within the serum, the AUC was not significantly different between SPAP for two and ten minutes and intravenous therapy (fig 3b). The peak concentration of gemcitabine within the serum was significantly higher after SPAP for two minutes compared to intravenous infusion (p = 0.004) (fig. 3a).

Flow reduction during SPAP for 50 and 90% did not result in a significant different lung (fig. 4a-b) and serum (fig. 5a-b) AUC compared to SPAP without flow reduction. However, the standard deviation of lung concentrations increased significantly as a higher flow reduction was installed (47%, 62% and 79% for normal flow, 50 and 90% flow reduction respectively) (fig 4a-b).
Liver concentrations of gemcitabine (11.4 ± 1.4 µg/g) and wet-to-dry ratios (8.3 ± 0.5) did not significantly differ between the five groups when determined at 45 minutes after the start of infusion.

Histologic examination of lung tissue after SPAP with gemcitabine suggests evidence of slight alveolar hyperplasia, which was more pronounced in the flow reduction groups with evident moderate congestion. No alveolar hyperplasia was present in the intravenous group (fig. 6). No abnormalities were observed in the slight sections of the pulmonary artery in either the SPAP or the intravenous group.

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Physiologic functions like heart rate, systemic and pulmonary blood pressure did not change significantly during and after SPAP.

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Discussion

To the best of our knowledge, this is the first report of a model of selective pulmonary artery perfusion without complete blood flow occlusion using an endovascular catheterisation technique that combined the properties of isolated lung perfusion and intravenous administration in order to improve the current treatment of NSCLC with minimal side-effects and to achieve down-staging of stage III a and b NSCLC. This approach resulted in significantly higher lung levels and equivalent serum and liver levels compared to the generally accepted intravenous route of cytostatic drug administration while physiological functions did not change.

This study evaluated infusion time of SPAP and pulmonary artery flow reduction during SPAP and compared the results to intravenous injection of gemcitabine. Clinically, gemcitabine is administered in a dose of 1 g/m^2 solved in 50 mL isotonic solution through an extremity infusion system during thirty minutes for the treatment of NSCLC. The infusion rate clinically applied, is limited due to the occurrence of local toxicity at higher rates. In this study, gemcitabine dose and solvent volume were equal in all groups.

All SPAP groups showed significantly higher lung AUC and peak levels compared to intravenous infusion. First, this observation is partially explained by a dilutional effect. In contrast to intravenous infusion, SPAP is characterized by infusion of the left or right pulmonary artery resulting in a two times higher blood concentration entering the treated lung. Furthermore, the blood concentration delivered at the tip of the SPAP catheter was increased by augmentation of the infusion rates resulting in six (SPAP, ten minutes) and thirty times (SPAP, two minutes) higher local blood concentrations compared to intravenous infusion. Therefore, SPAP resulted in significantly higher peak pulmonary concentrations and areas under the curve. Second, the high pulmonary peak and AUC levels during and after

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SPAP are explained by the important first-pass capacity of the lung resulting in systemic plasma AUC levels that are in the same range compared to intravenous infusion. However, pulmonary peak concentrations after SPAP for two minutes without blood flow occlusion were only five times higher compared to intravenous infusion while local blood concentrations delivered at the tip of the catheter during SPAP were thirty times higher. Saturation of the first-pass capacity of the lung at two minutes infusion time or a too short uptake interval are the most reasonable explanations.
Interestingly, SPAP for ten minutes resulted in either six times higher lung ánd local blood levels delivered at the catheter tip suggesting that lack of first-pass saturation is present. Furthermore, pulmonary AUC levels were significantly higher compared to SPAP for two minutes. To our opinion, SPAP for ten minutes seems to be the most efficient strategy in saturating the lung, because the time interval of the peak concentration after SPAP for two minutes is too short to achieve intracellular saturation.
Important first-pass capacity of the lung was shown in a former rat study in our laboratory (Van Putte et al., 2003[b]). Significantly higher gemcitabine lung levels were achieved after pulmonary artery perfusion without control of the pulmonary veins while significantly less systemic toxicity was observed compared to intravenous injection of even a higher dose during the same infusion interval (Van Putte et al., 2003[b]).

Reduction of blood flow during SPAP up to 50 and 90% was applied and compared to normal blood flow in order to investigate the relation between blood flow and cytostatic drug uptake into the lung. In contrast to what we expected, SPAP with 50% flow reduction resulted in even lower pulmonary drug levels compared to normal blood flow while the blood concentration delivered at the catheter tip was even twice as high. Furthermore, 90% flow reduction resulted in higher levels compared with normal blood flow. Obviously, flow

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reduction during SPAP resulted in increasing standard deviations (47%, 62% and 79% for normal flow, 50 and 90% flow reduction respectively). We hypothesize that this phenomenon may be explained by compensatory pulmonary vasoconstriction during flow reduction in order to maintain pulmonary artery pressure finally resulting in inhomogeneous distribution of the drug infused.

Three former studies evaluated feasibility of endovascular pulmonary artery perfusion for the treatment of pulmonary metastases using adriamycin and cisplatin. These studies aimed to achieve higher local pulmonary drug levels and less systemic toxicity in order to treat pulmonary metastatic disease more aggressively. In contrast, we believe that this endovascular method is an ideal strategy to treat local NSCLC and lymph node metastases more intensively while furthermore treating the systemic disease in an equivalent manner as intravenous therapy does. Furthermore, these studies are of limited significance probably due to inhomogeneous drug distribution.

First, Karakousis et al. performed Schwann-Ganz catheterisation of lobe branches separately under fluoroscopic control in seven patients with recurrent pulmonary metastases who had already received the maximum dose of adriamycin (Karakousis et al., 1981). They received a dose of 10-20 mg diluted in 50 mL infused over a 1- to 2-minute period followed by five minutes of blood flow occlusion. A total of fifty-six injections were given in lobar arteries in the seven patients treated. After metastasectomy, three patients were disease-free after four months while the other patients had tumour progression. The disappointing results in this study can be explained by some major limitations. First, the dose and infusion rate are not standardised between the patients resulting in high variability of the intravascular drug concentration delivered. Furthermore, they applied blood flow occlusion during infusion that should have resulted in inhomogeneous distribution as shown in our study. During each

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injection, only one of different lobar branches was selected resulting in varying pulmonary distribution volumes and therefore varying local tissue levels (Karakousis et al., 1981).
Second, Furrer et al. compared endovascular pulmonary artery perfusion during blood flow occlusion with isolated lung perfusion and intravenous infusion of doxorubicin (Furrer et al., 1998). Both selective techniques resulted in significantly higher lung concentrations and lower serum levels compared to intravenous administration. However, pulmonary artery perfusion was performed during blood flow occlusion that should have resulted in inhomogeneous distribution. Furthermore, they achieved lower serum levels compared to intravenous injection while we believe that maximal systemic exposure during SPAP is essential to treat (micro)metastatic disease outside the lungs in lung cancer (Karakousis et al., 1981).

Third, Brown et al. recently published their results on selective pulmonary artery perfusion during blood flow occlusion compared to intravenous infusion of cisplatin using a swine model (Brown et al., 2006). They concluded that no relation was observed between the infusion time and inflow concentration compared to the final lung tissue concentrations. They showed a very wide range (6.63-76.78 fmol/µg) of final lung concentrations probably due to inhomogeneous drug distribution as shown in our study in a pulmonary blood flow reduction experiment (fig. 3a) (Brown et al., 2006).

However, based on this paper we modified the hypothesis from treating lung metastases using this catheter technique towards the hypothesis to achieve down-staging of the T and N status of primary lung cancer. In our study we showed high peak concentrations in lung tissue after SPAP that were significantly higher compared to the intravenously treated animals suggesting (not tested in this study) that more efficacy could be achieved.

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Our study furthermore shows a rapid washout phenomenon after peak pulmonary concentrations were achieved at the end of the SPAP procedure (figure 2a and 4a). In a very recent rat study, we reported that a major part of the drug taken up during isolated lung perfusion is quickly exchanged into the circulation during the washout interval and during reperfusion (Van Putte et al., 2006). Main part of the drug is returned from the interstitium into the vascular compartment based on simple diffusion suggesting that part of the drug did not enter the cells due to a too short uptake interval. In the same article, we achieved stabilization of high lung peak levels after isolated lung perfusion by delayed restoration of normal blood circulation up to thirty minutes (Van Putte et al., 2006). Further studies are necessary to confirm these findings in this endovascular SPAP model.

Patients suffering from NSCLC die due to local pulmonary or distant recurrences in 25 % and 75% respectively (Rivera et al., 2001). In fact, in 25% of these patients, local control is not achieved after initial surgical and cytostatic treatment.

In this study, we created a model of endovascular SPAP as a hybrid modality for the treatment of patients suffering from NSCLC to treat the primary tumour (T status) and pulmonary lymph nodes (N status) more aggressively in order to improve the current treatment of NSCLC with minimal side-effects and to achieve down-staging of stage III a and b NSCLC . This technique is characterized by the superior pharmacokinetic properties of isolated lung perfusion resulting in high local lung and lymph node drug levels and by the properties of intravenous infusion in order to achieve total body exposition. As shown in figure 2 and 3, significantly higher lung levels were achieved after SPAP while plasma and liver levels did not significantly differ compared to intravenous infusion.

Interestingly, a significant portion of both lymph node and pulmonary tumour vasculature is

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fed by the pulmonary arterial circulation (Mochizuki et al., 2007, Miller et al., 1967). Therefore, patients suffering from stage 1,2 and 3 NSCLC will be the main target population for treatment with endovascular SPAP. First, SPAP aims to result in primary tumour reduction (T status) before surgical treatment. Second, SPAP results in higher drug exposure of the residual lobes and of local lymphogenic metastatic disease (N status) in order to achieve down-staging of stage III NSCLC towards a surgical stage. Third, systemic serum and liver levels equivalent to intravenous therapy will treat systemic disease in the same way as achieved in currently applied intravenous schedules for NSCLC.

The currently applied intravenous schedule for the treatment of NSCLC consists of a combination of gemcitabine and cisplatin resulting in synergistic activity (Peters et al., 2006, Bergman et al., 1996). Future studies have to find out if co-administration of platin-based drugs to this SPAP gemcitabine model will be feasible. Subsequently, these results have to be validated in a phase 1 study due to the lack of large animal tumour models.

In conclusion, we created an endovascular SPAP model that results in higher local pulmonary drug levels with equivalent serum and liver concentrations compared to intravenous infusion. This new approach could potentially improve prognosis of NSCLC by reducing the primary tumour size and by down-staging of the lymph node status.

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Acknowledgements

The authors thank dr. Ellen Tromp, clinical epidemiologist, for careful reviewing statistics. The authors furthermore acknowledge Cees Verlaan, Maringa Emons and Merel Schurink for excellent biotechnical support.

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Figure legends.

Figure 1a. Experimental setting.

Figure 1b. The tip of the catheter was positioned in the left main stem pulmonary artery just proximal of the first side-branches. A 12 mm flow probe was placed around the left pulmonary artery for blood flow measurements just distal of the tip of the balloon catheter and just proximal of the first side branches.

Figure 2a. Median AUC levels of gemcitabine lung concentrations (± standard error) of standard intravenous infusion during thirty minutes, SPAP two and SPAP ten minutes. Both SPAP groups resulted in significantly higher AUC ($p = 0.001$) compared to intravenous infusion while SPAP ten minutes resulted in the highest AUC ($p = 0.045$ compared to SPAP two minutes)

Figure 2b. Median gemcitabine lung concentrations (± standard error) of standard intravenous infusion during thirty minutes, SPAP two and SPAP ten minutes in function of time. SPAP two minutes resulted in the highest peak concentration at two minutes ($p = 0.01$ compared to intravenous infusion).

Figure 3a. Median AUC levels of gemcitabine serum concentrations (± standard error) of standard intravenous infusion during thirty minutes, SPAP two and SPAP ten minutes. No significant differences in AUC were observed between the three groups.

Figure 3b. Median gemcitabine serum concentrations (± standard error) of standard intravenous infusion during thirty minutes, SPAP two and SPAP ten minutes in function

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of time. The median peak concentration of SPAP two minutes was significantly higher compared to intravenous infusion (p = 0.004).

Figure 4a. Median AUC levels of gemcitabine lung concentrations (± standard error) of SPAP during two minutes without, with 50% and 90% pulmonary blood flow reduction. No significant differences were shown between the three groups. However, standard error increased with augmentation of blood flow reduction suggesting compensatory vasoconstriction and therefore inhomogeneous distribution during balloon inflation.

Figure 4b. Median gemcitabine lung concentrations (± standard error) of SPAP during two minutes without, with 50% and 90% pulmonary blood flow reduction. No significant differences were shown between the three groups.

Figure 5a. Median AUC levels of gemcitabine serum concentrations (± standard error) of SPAP during two minutes without, with 50% and 90% pulmonary blood flow reduction.

Figure 5b. Median gemcitabine serum concentrations (± standard error) of SPAP during two minutes without, with 50% and 90% pulmonary blood flow reduction. No significant differences are depicted between the three curves.

Figure 6. Histologic findings of the experimental groups on H&E staining: intravenous infusion (A), SPAP 2 minutes (B), SPAP 50% flow reduction (C), and SPAP 90% flow reduction (D).

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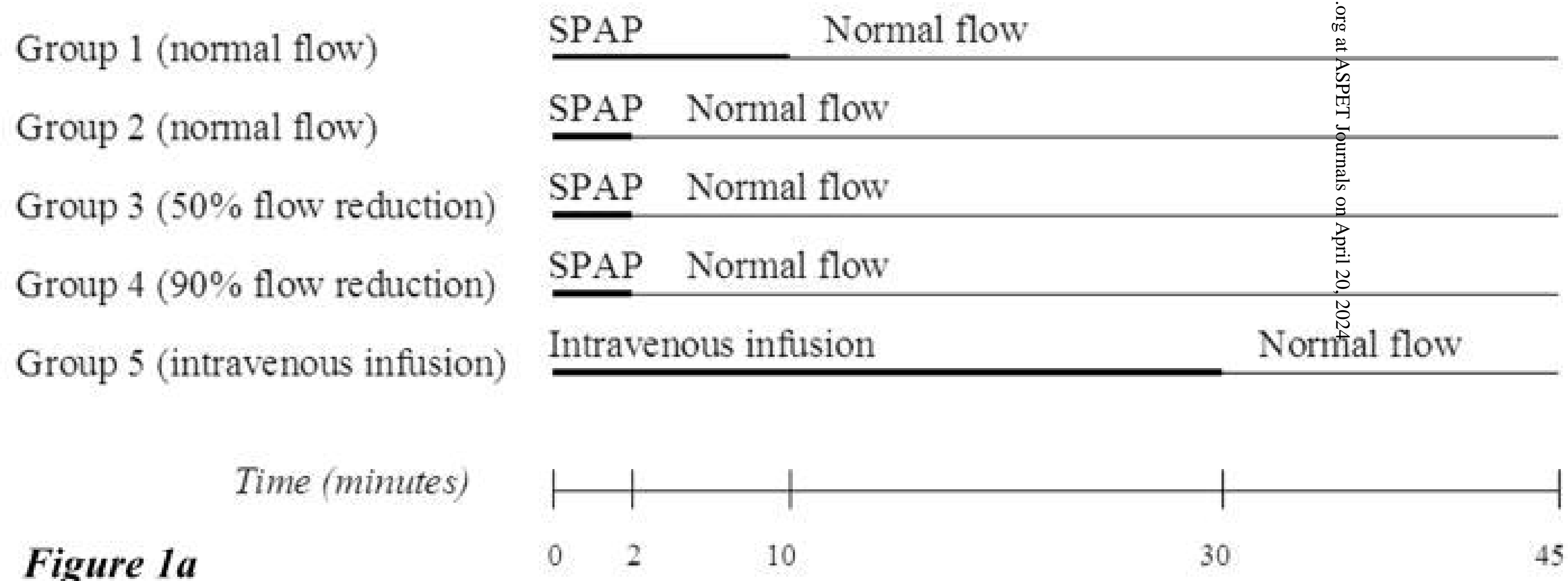

Figure 1a

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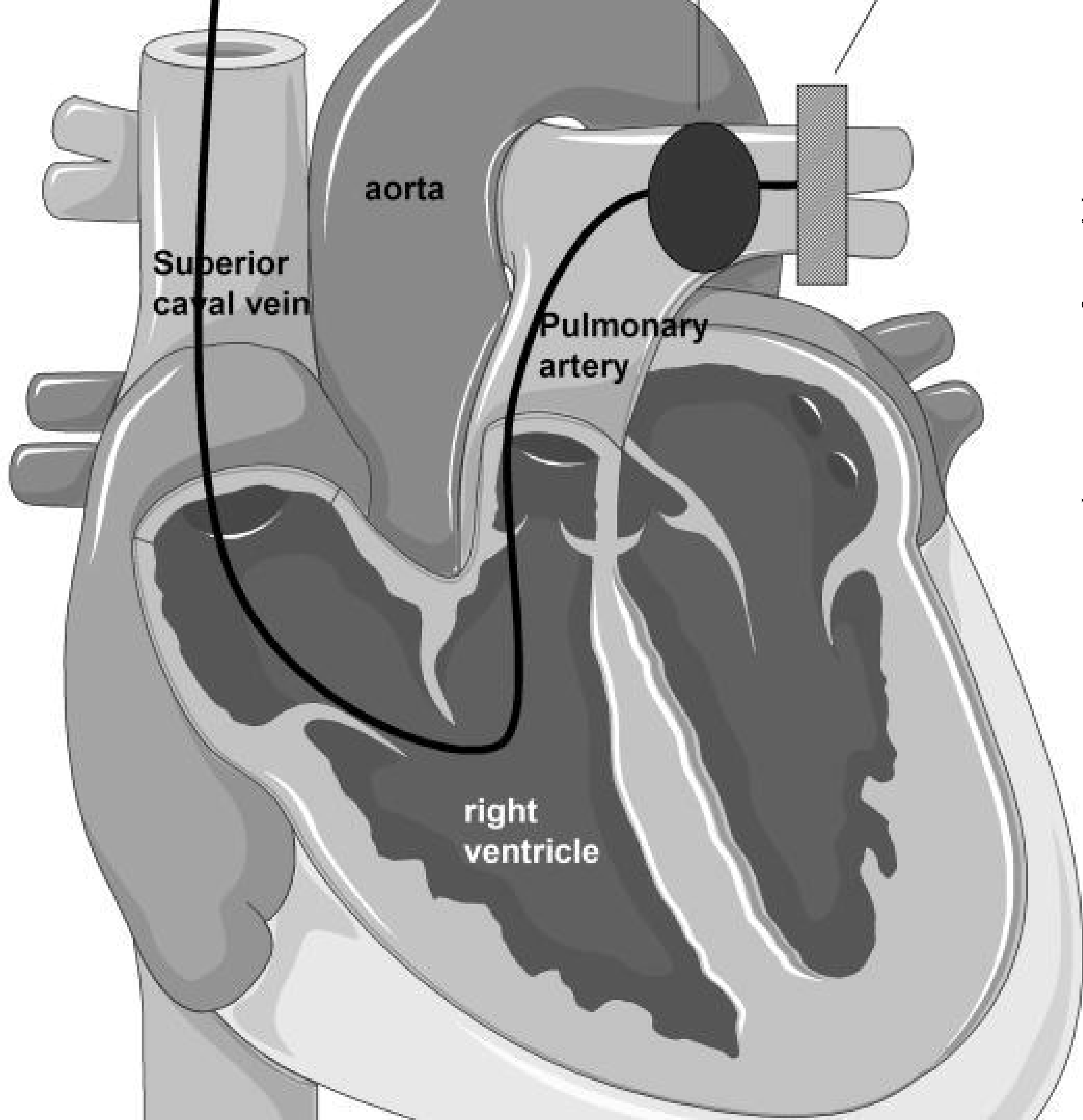

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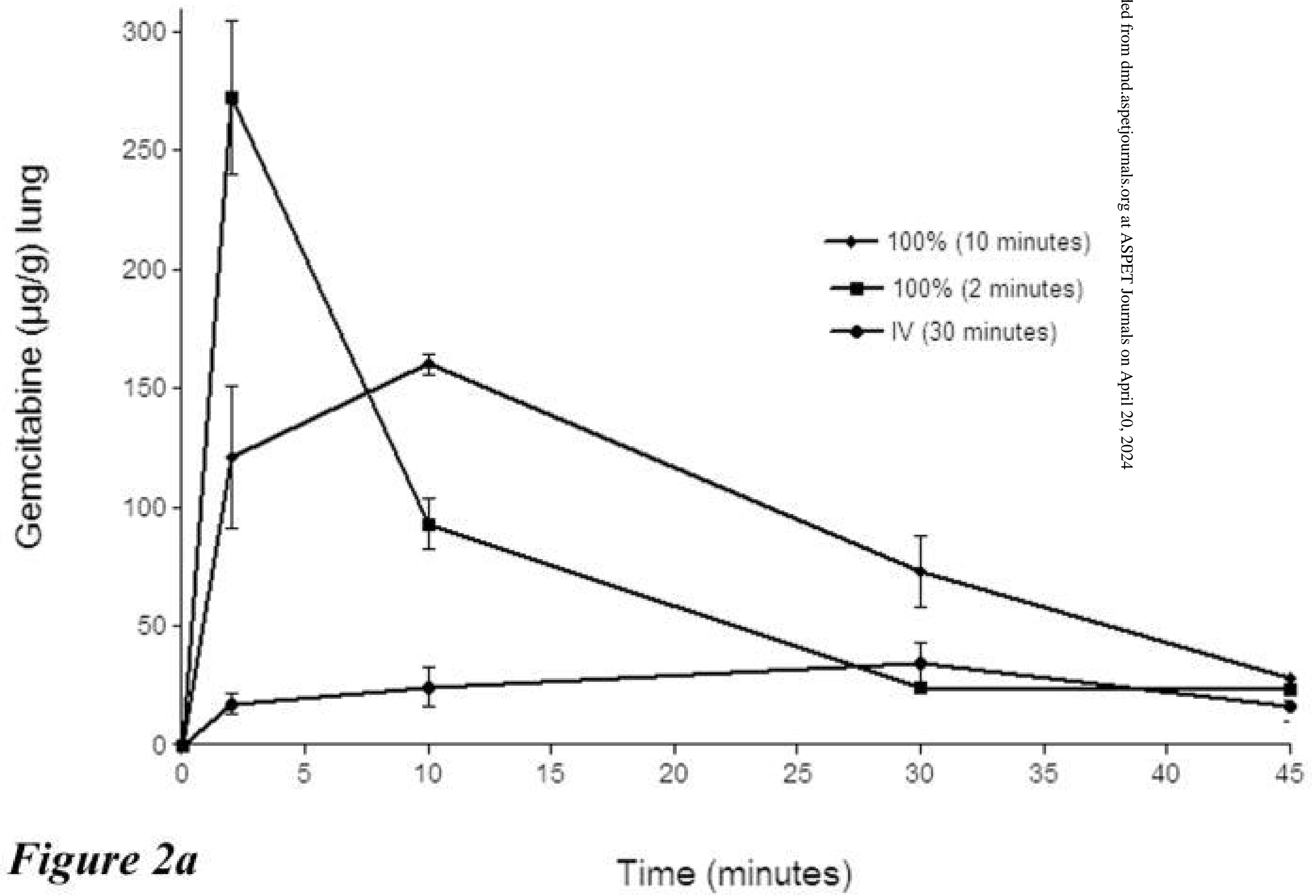

Figure 2a

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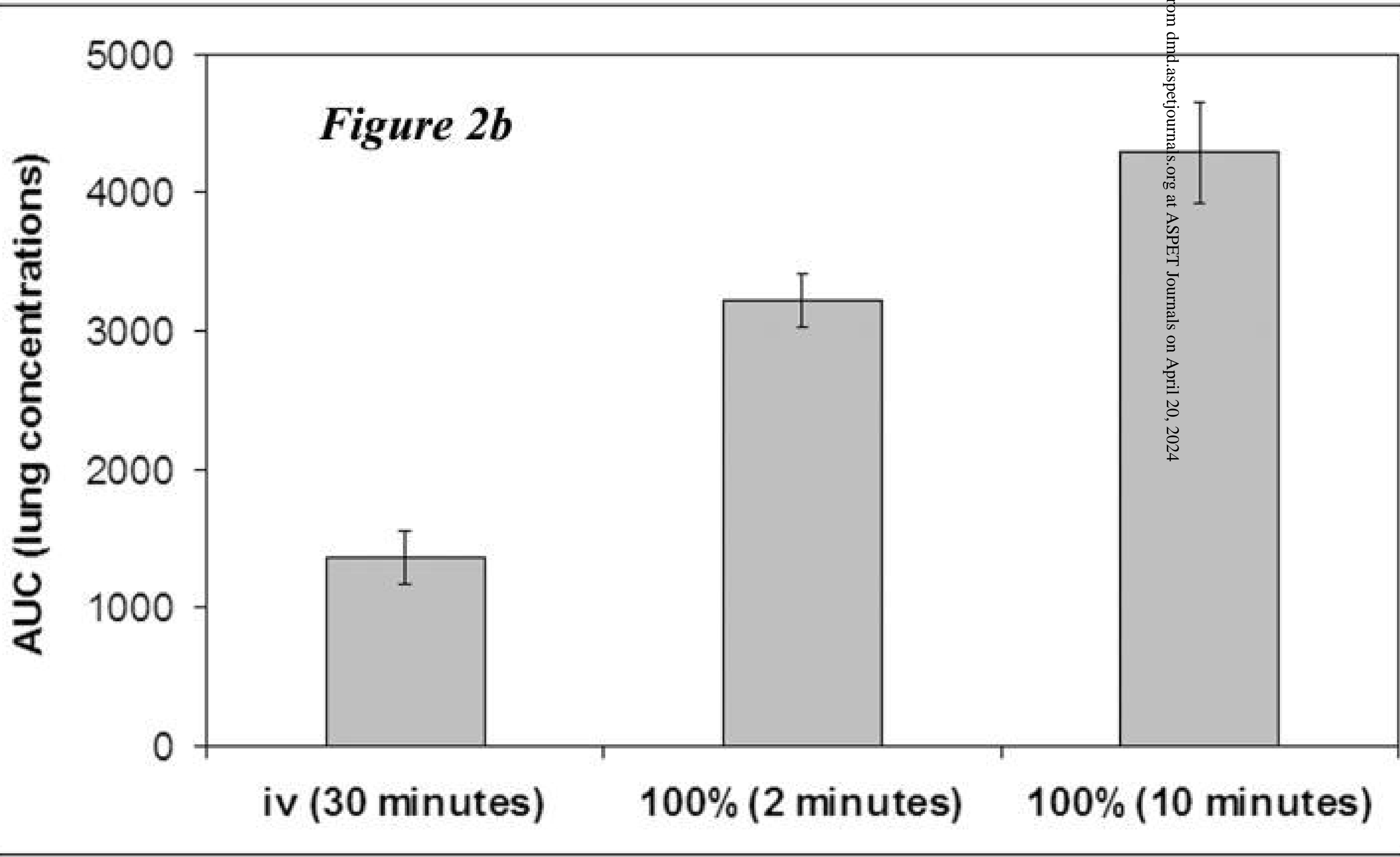

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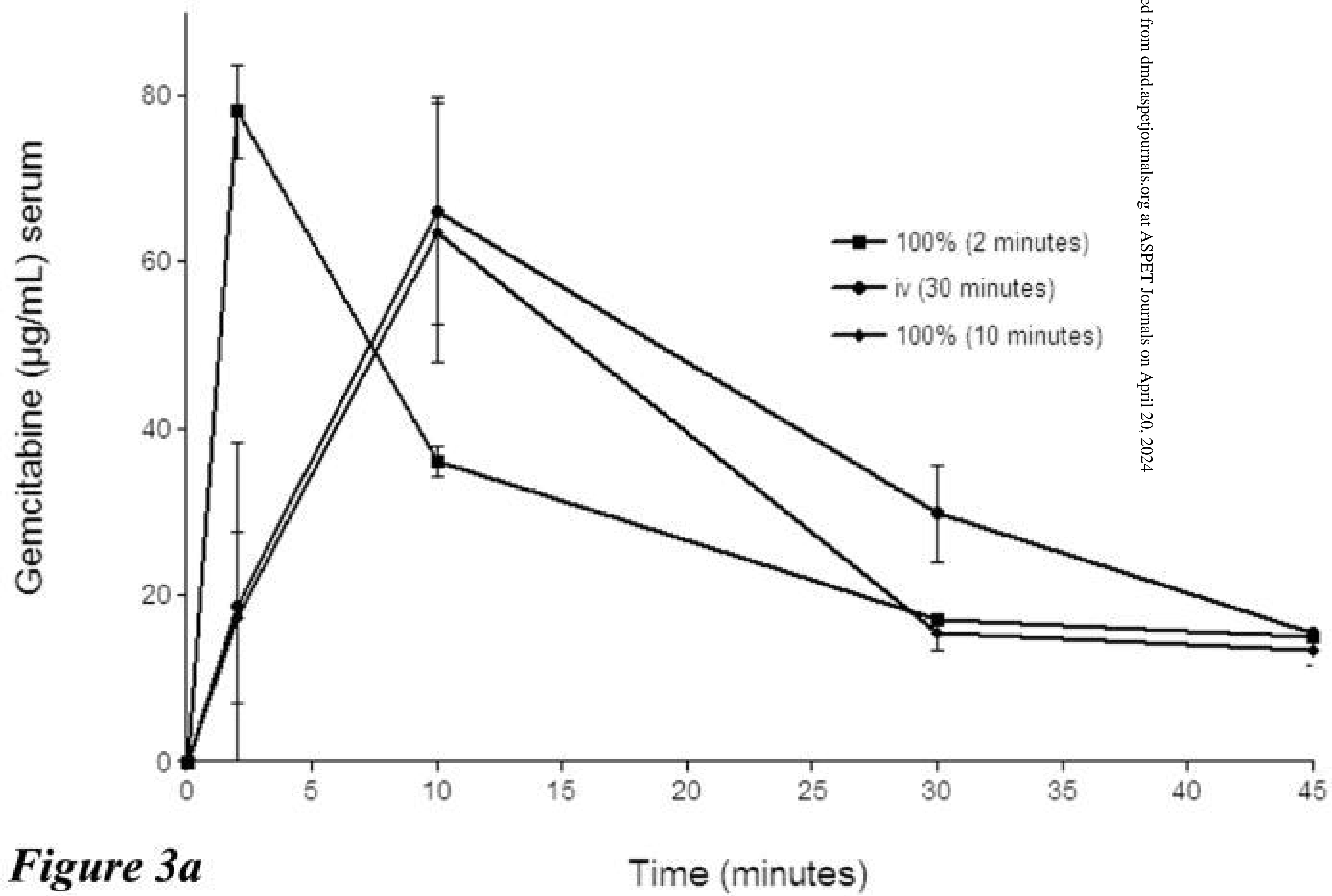

Figure 3a

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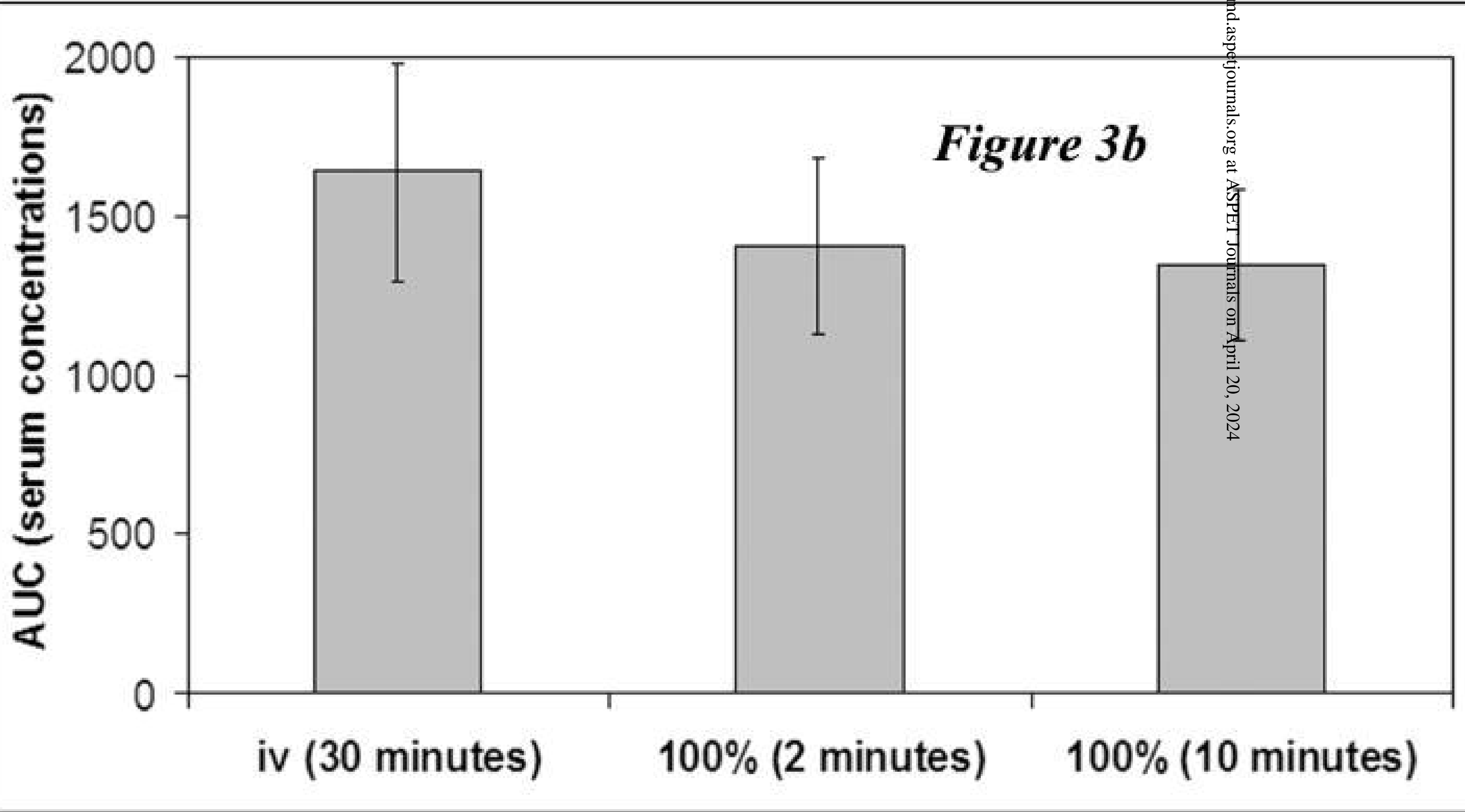

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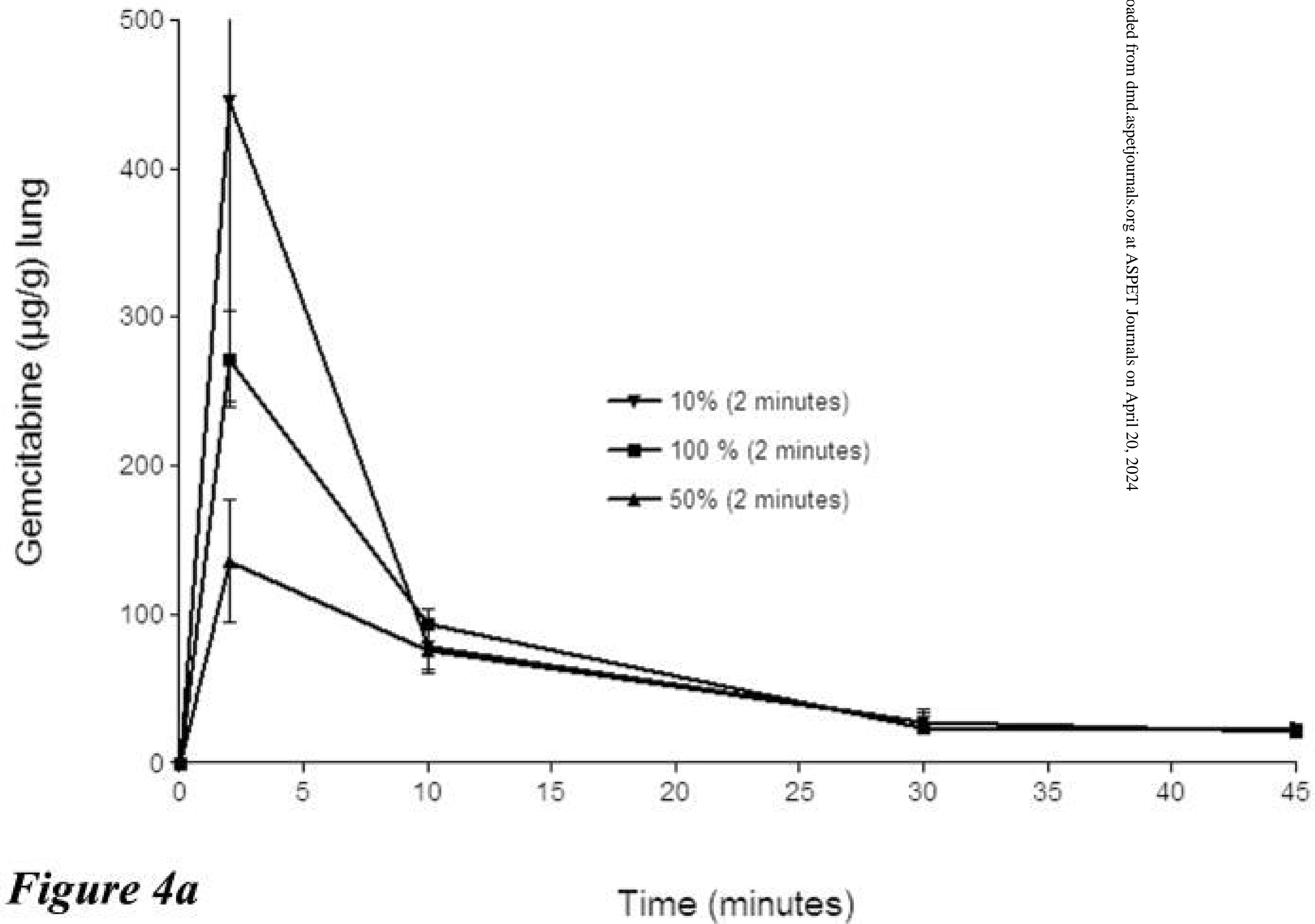

Figure 4a

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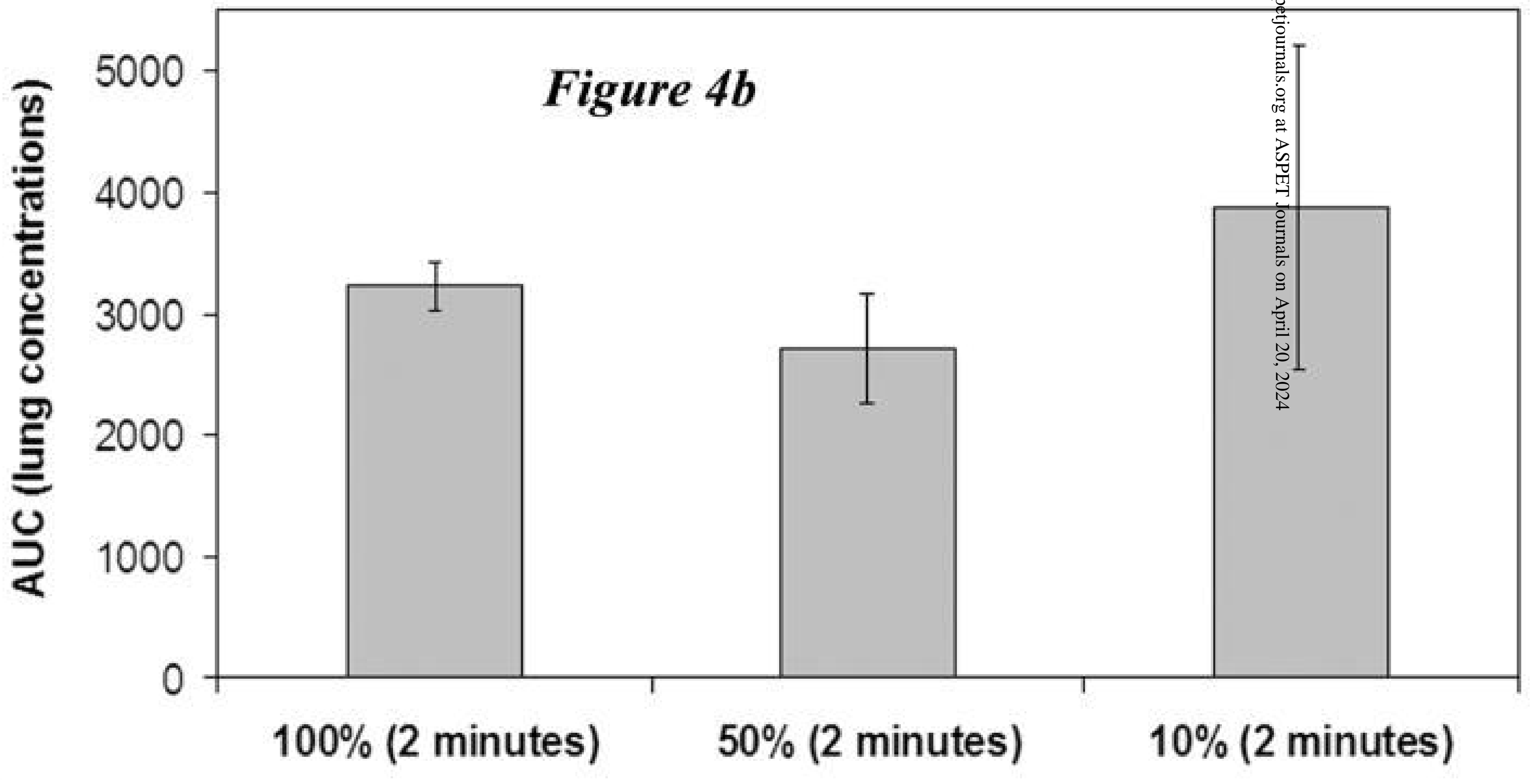

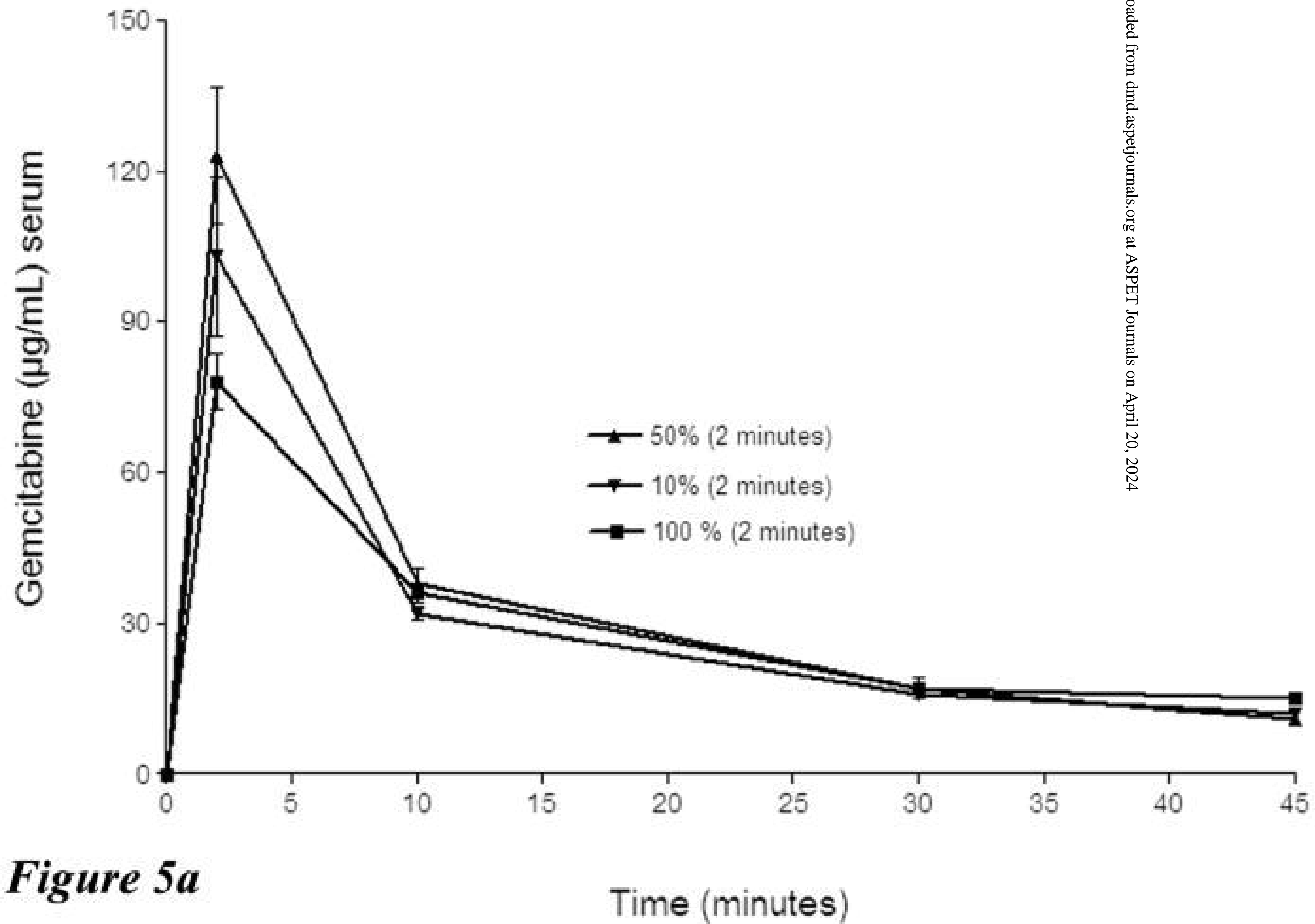

Figure 5a

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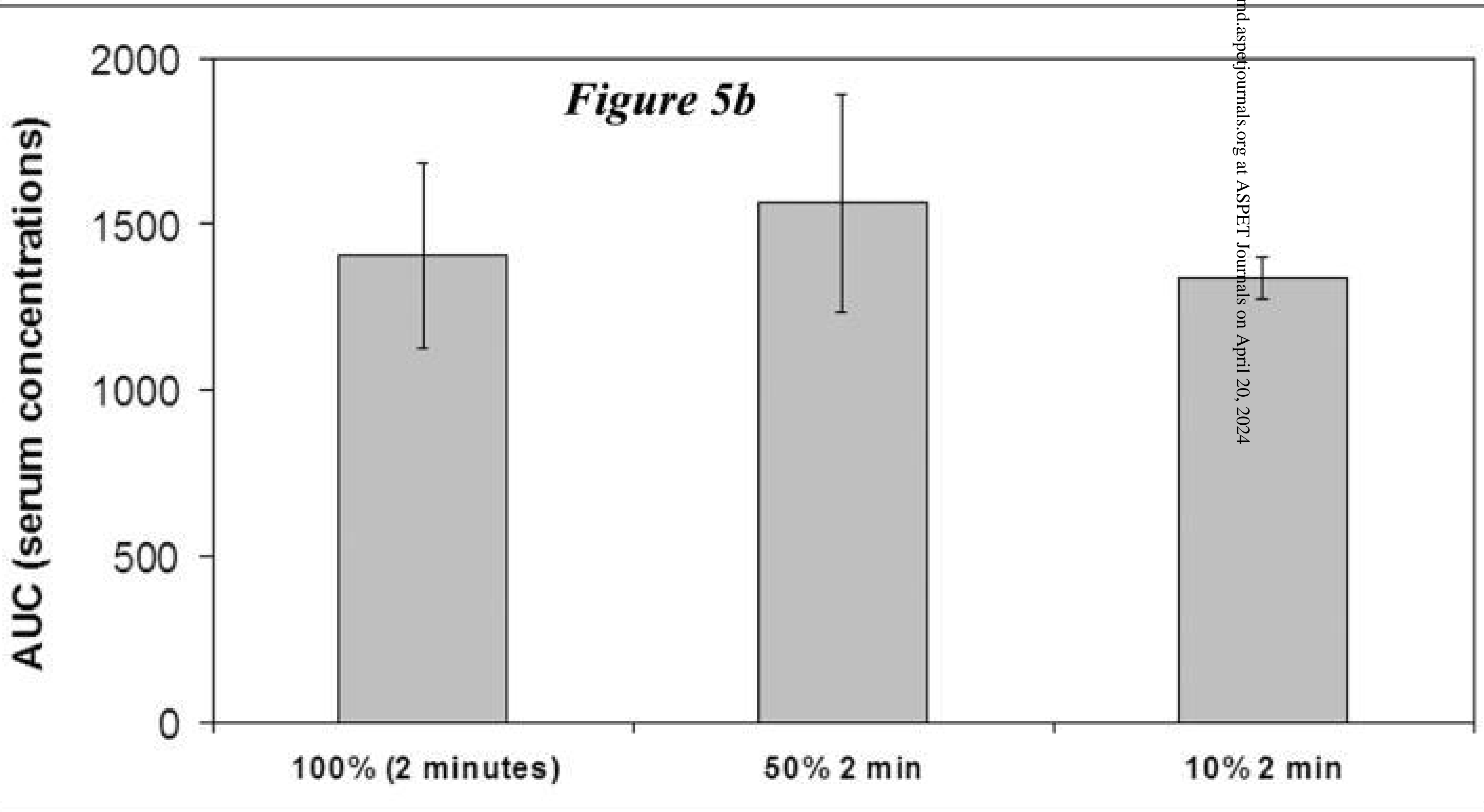

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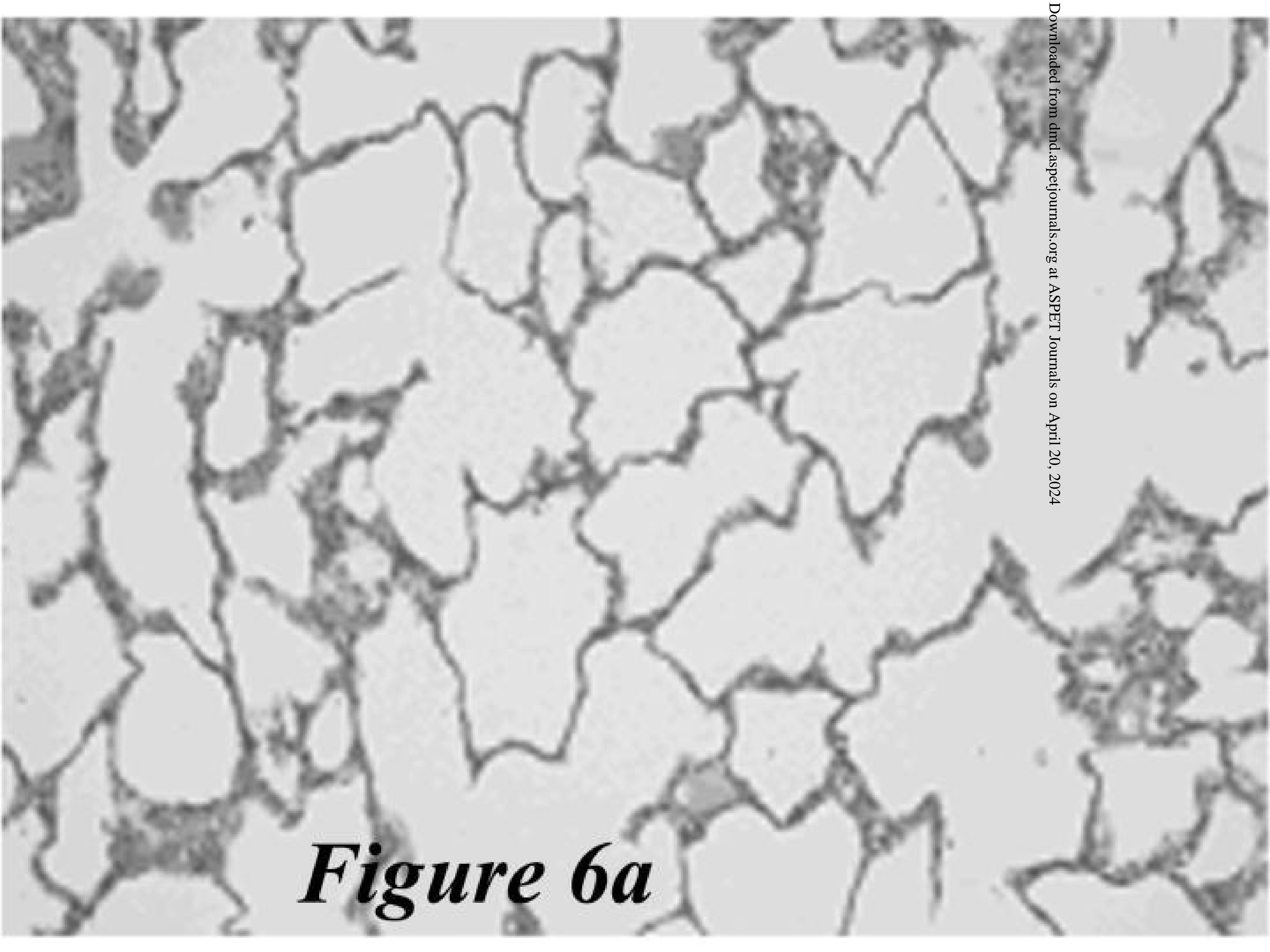

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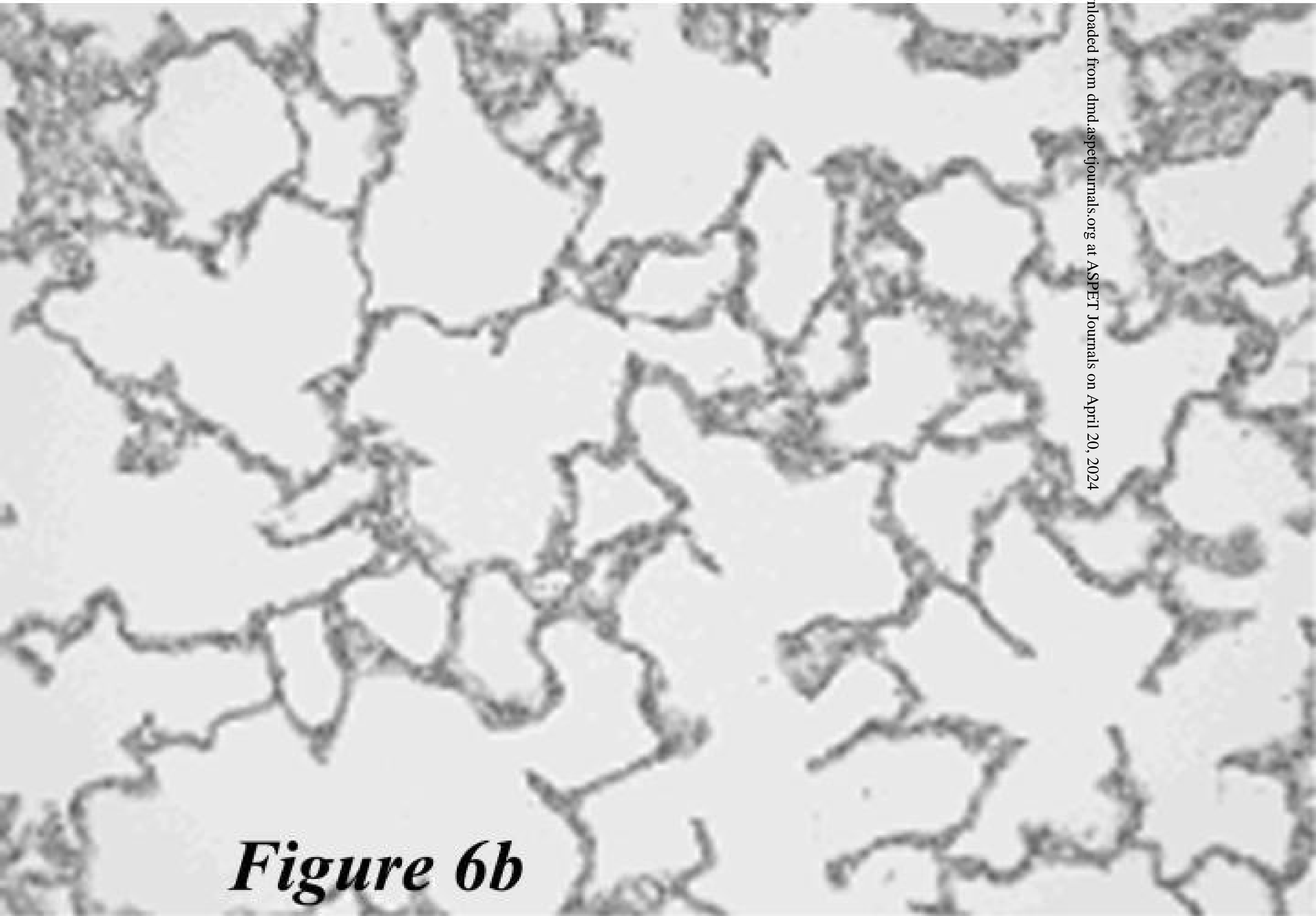

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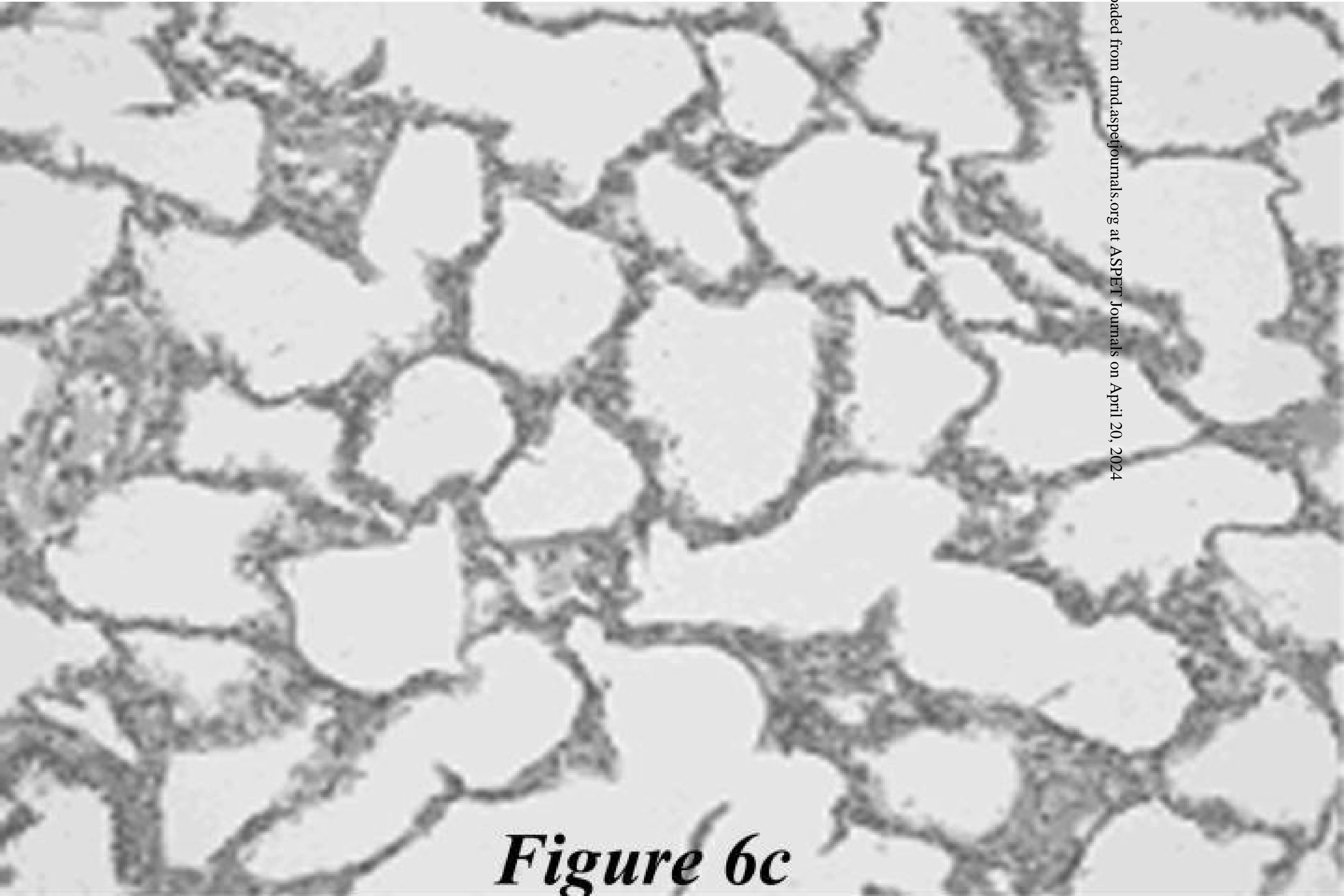

Figure 6c

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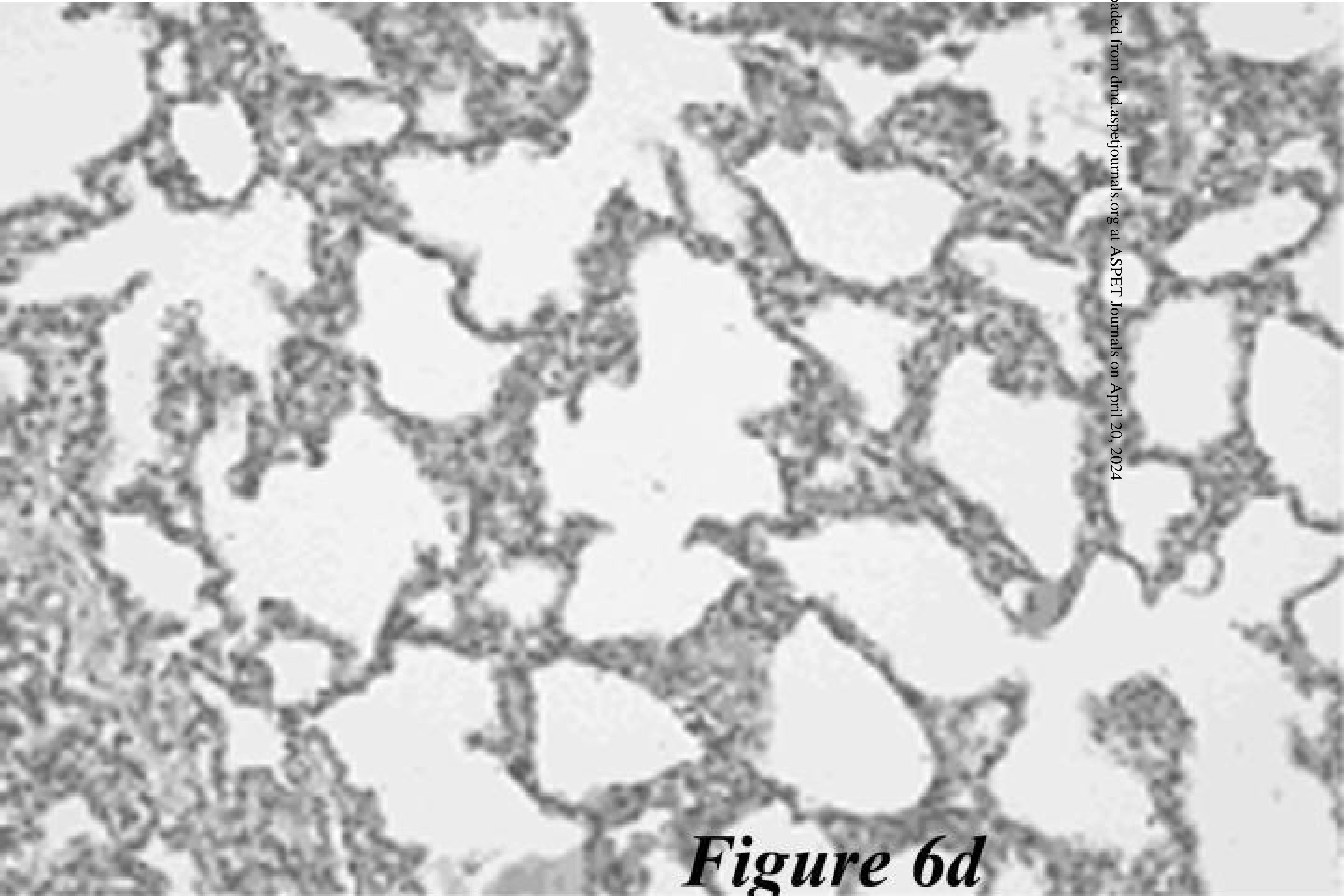

Figure 6d

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