Acta Orthop. Belg., 2012, **78**, 506-511

ORIGINAL STUDY

Safety of retransfusing shed blood after local infiltration analgesia in total knee arthroplasty

Bregje J. W. Thomassen, Laurens Pool, Rudolf Van Der Flier, Rudolf Stienstra, Bastiaan A. in 't Veld

From the Medical Center Haaglanden, The Hague, The Netherlands

We investigated the safety of LIA (local infiltration analgesia) combined with retransfusion of drained blood. Total knee arthroplasty patients received two peri-articular injections during surgery followed by continuous infusion, both with ropivacaine (567 mg). Ropivacaine plasma concentrations were determined in blood samples taken at 0, 3, 6 and 24 hours postoperatively. The collected shed blood was not retransfused, instead retransfusion was modelled by estimating the cumulative plasma concentrations at 6 hours postoperative. Total and unbound ropivacaine plasma concentrations ranged respectively from 0.08 to 1.9 mg/L and 0.003 to 0.11 mg/L. An average of 13.1 ± 3.7 mg unbound ropivacaine would have been returned to the patient. The estimated cumulative ropivacaine plasma levels showed that instant retransfusion would have led to plasma levels below 0.26 mg/L. It appears to be safe to transfuse autologous blood in combination with LIA. However, before drawing definite conclusions formal measurement of actual concentrations is required.

Keywords : local infiltration analgesia ; retransfusion drain ; total knee arthroplasty ; ropivacaine.

INTRODUCTION

Recovery after total knee arthroplasty (TKA) is hampered by postoperative pain when not adequately dealt with (*20*). A multimodal approach that reduces nociception is recommended to achieve maximum efficacy in pain control. As part of a 'multimodal approach' local infiltration technique with local anaesthetics, local infiltration analgesia (LIA), has been introduced. Large case series have been performed with satisfactory results for pain relief and despite the high volume, the frequency of side effects of the local infiltration techniques was low (*13,17*).

Several LIA variations have been described in the last decades, with different routes of administration, types of anaesthetics, and the combination of intraoperative infiltrations with postoperative catheters. At the moment there is non-uniformity with regard to the preferred technique. Kohan and Kerr described their LIA technique using a catheter for postoperative single-shot local anaesthetic administration after 15 to 20 hours (*13*). Bianconi *et al*

■ Bregje J. W. Thomassen, MSc, Research Coordinator.
■ Laurens Pool, MD, Resident in Orthopaedic Surgery.
■ Rudolf van der Flier, MD, Orthopaedic Surgeon.
Department Orthopaedic Surgery, Medical Center Haaglanden, The Hague, The Netherlands.
■ Rudolf Stienstra, MD, PhD, Anaesthesiologist.
Department of Anaesthesiology, St. Maartenskliniek, Nijmegen, The Netherlands.
■ Bastiaan A. in 't Veld, MD, PhD, Anaesthesiologist.
Correspondence : Bregje J. W. Thomassen, Department Orthopaedic Surgery, Medical Center Haaglanden, PO Box 432, 2501 CK The Hague.
E-mail : b.thomassen@mchaaglanden.nl

Conflict of interests : The laboratory analyses were performed by the Pharmacy Hague Hospitals. This study was partly funded by an unrestricted grant from AstraTech, Mölndal, Sweden and Baxter Inc, Europe. They did not participate in the conduct of the study design or analysis.

favoured the LIA technique when compared to systemic analgesia because of less postoperative pain, opioid use reduction and shorter hospital stay (*3*).

The effectiveness of autologous retransfusion drains has been studied by several authors. Moonen *et al* and Cheng *et al* found a reduction in allogeneic blood transfusions in knee- and hip arthroplasty (*7,16*). However, Amin *et al* and Abuzakuk *et al* found no effect of retransfusion drains (*1,2*). Despite the discussion about the effectiveness of wound drains in general, in our hospital, as in many clinics worldwide retransfusion of shed blood is still standard care and independent of the haemoglobin level in many hospitals.

A combination of LIA and retransfusion of shed blood involves the risk of reinfusing potentially high concentrations of local anaesthetics. Ropivacaine (Naropin®, AstraZeneca) is most frequently used in LIA, because of its enhanced safety profile compared to other long-acting local anaesthetics (*12,25*). Parker *et al* reported the safe combination of both techniques in case of single shot LIA. However, no continuous wound catheter was present and no unbound ropivacaine plasma concentrations were analysed (*19,20*). Most of the plasma binding of local anaesthetics is due to association with α-1-acid glycoprotein (AAG). In this case, unbound ropivacaine plasma concentration interacts with receptors to produce pharmacological or toxicological effects after systemic administration. That is why the unbound ropivacaine plasma concentration is of particular importance since side effects and complications are largely attributable to this fraction.

This study was designed to determine ropivacaine plasma concentrations in the patient and in shed blood after local (continuous) infiltration and to estimate cumulative ropivacaine plasma concentrations assuming retransfusion of shed blood.

PATIENTS AND METHODS

This experimental prospective study protocol was approved by the local Medical Ethics Committee (CCMO no. NL25137.098.08) and registered in the Dutch trial registry (NTR1784). After written informed consent had been obtained, twenty primary TKA's were enrolled in the study. Inclusion criteria were age from 18 till 90 years, ASA I-II, haemoglobin (Hb) levels above 7.5 mmol/L and normal renal function (Modified diet in renal disease equation (MDRD)) value above 48mL/min. Before surgery two intravenous cannulae were inserted into a vein of both arms, one for routine monitoring of the patient during surgery and one (controlateral side) in order to obtain blood samples. All patients received lumbar spinal anaesthesia with 15 mg bupivacaine 0.5%. Two orthopaedic surgeons performed the operations according to standard procedure. Cemented cruciate retaining components (PFC Sigma, DePuy, Johnson&Johnson) were placed and patella resurfacing was done when necessary.

A solution consisting of 50 mL ropivacaine 0.75% (= 375 mg) (AstraZeneca, Sweden), 50 mL NaCl 0.9% and 0.5 mL of 1:1000 epinephrine was injected in the peri-articular tissues. The solution was equally divided in two 50 mL syringes and injected with 22 gauge spinal needles. Before placement of the final implants the posterior capsule and deep peri-articular tissues were infiltrated with the first syringe. The second syringe was used to infiltrate the synovium of the suprapatellar pouch, quadriceps and patellar tendons and subcutaneous tissue surrounding the incision.

Additionally, near the end of the operation the Solace™ Infusion System (Apex medical, San Diego) was inserted and connected. This single use elastomeric type pump was filled with 270 mL ropivacaine 0.2% without epinephrine, which was attached to two small catheters, each producing 2 mL/h, which were placed intraarticular and subfascial. A total of 96 mL ropivacaine 0.2% (= 192 mg) was infused during the first 24 hours after surgery.

Furthermore, a Bellovac autologous blood transfusion (ABT) drain (AstraTech, Sweden) was placed intraarticularly.

Blood samples were taken at the conclusion of surgery (T = 0) before the Solace infusion system and drain were opened and 3, 6 and 24 hours after the first sample. At 6 hours, the drain was disconnected and two samples were taken out of the blood bag : one before and one after passage through the filter cascade. Shed blood was not returned to the patient. The 6 hour sampling time was taken because this is the maximum time allowed for blood collection in autologous retransfusion drains.

All samples were centrifuged at 3000 rpm for 10 minutes and plasma was stored at minus 80 degrees Celsius. The wound drain and Solace infusion catheters were removed after 24 hours.

During hospitalisation haemoglobin levels (Hb) were measured preoperatively and postoperatively on day 1 and 3.

Analyses

Total and unbound ropivacaine concentrations were measured with liquid chromatography-mass spectrometry (LC-MSMS, Agilent Technologies 6410 Triple Quad) *(4)*. The accuracy and intermediate precision of these analyses were respectively 2.2-4.4% and 2.0-2.9%.

The free ratio of ropivacaine for each patient (Psfr) was calculated by dividing the unbound concentration by the total concentration.

Subsequently we modelled the theoretical maximum unbound ropivacaine plasma concentration if the shed blood would have been instantly returned to the patient. We estimated circulating plasma volume per patient according to the formulae of Lemmens *et al (15)* and the patient specific haematocrit (preoperative) values. Next, we estimated the theoretical maximum unbound ropivacaine plasma concentration as follows :

((Rbel × Vbel × $Psfr_6$) + (Rpl_6 × pat specific circulating plasma volume)) / (pat specific circulating plasma volume + Vbel). (Rbel = unbound ropivacaine concentration in mg/L in the retransfusion device after blood passage through the filter cascade, Vbel = volume of shed blood in L, $Psfr_6$ = patient specific free ratio of ropivacaine at 6 hours postoperatively, Rpl_6 = unbound ropivacaine plasma concentration in mg/L at 6 hours postoperatively)

In this calculation we assume that a part of the unbound ropivacaine in the shed blood will instantly bind to AAG upon retransfusion, the amount of ropivacaine remaining unbound is determined by the $Psfr_6$.

Statistics

The continuous data are presented as the number of subjects, mean, standard deviation (SD), minimum and maximum values, and categorical data expressed as frequencies and percentages. Normally distributed data are presented as mean ± SD ; in case of non-normal distribution median and range will be used.

Spearman's correlations were calculated to examine a potential relationship between patients' characteristics (age, BMI and renal clearance) and ropivacaine concentrations in plasma. Data were analysed using SPSS 17.0 (SPSS for Windows, Chicago : SPSS Inc.).

RESULTS

Twenty consecutive eligible patients undergoing elective TKA were enrolled in the study (Table I. general characteristics). Patelle resurfacing was performed in 11 patients. The first and second local analgesia block were given respectively 43 and 63 minutes (on average) after start of surgery (SD : 10 min). The catheter for continuous infusion was unclamped 57 minutes (SD : 11 min) after the first block was given. The first sample was taken 34 minutes (SD : 9 min) after the second intraoperative injection.

Mean total and unbound ropivacaine plasma concentrations are shown in Table II, on average the free fraction of ropivacaine was 4.8% (SD : 0.7%).

The plasma values at 6 hours postoperatively ranged for total ropivacaine from 0.54 to 1.69 mg/L and unbound ropivacaine 0.03 to 0.11 mg/L at 6 hours postoperatively. The C_{max} (peak concentration) of the total ropivacaine plasma concentration was 1.89 mg/L found at 24 hours postoperatively, for unbound ropivacaine plasma concentration C_{max} was 0.11 mg/L at 6 hours postoperatively. Both values were found in different patients. The C_{max} for the total ropivacaine plasma concentration was found in 13 patients at 24 hours postoperatively, others had their maximum at 3 hours (n = 2) and 6 hours (n = 5) postoperatively. The C_{max} for unbound ropivacaine plasma concentration was found in half of the patients at 6 hours, 5 patients had C_{max} at 3 hours and 5 patients at 24 hours postoperatively.

A negative correlation was found only between age and BMI (p = -0.458) and the differences in C_{max} time points. Furthermore, no significant relationship was found between the time window, the single-shots and the sampling points.

The median shed blood volume was 600 mL (range : 303-869 mL). In one case the exact amount could not be measured, the missing value was imputed from the mean volume of the shed blood in all other patients, which was 591 mL.

There was a small difference in the total and unbound ropivacaine concentration before and after filtration of the shed blood, respectively 33.05 vs. 32.7 mg/L for the total concentration. We used the filtered total ropivacaine concentration for model-

Table I. — Patient demographic data

	Mean ± SD (range)
Age (years)	71.3 ± 7.5 (58-84)
Body Mass Index (kg/m²)	27.1 ± 3.5 (21-37)
Renal clearance (MDRD) (mL/min)	69.7 ± 11.8 (48-86)
Male / female	15 (75%) / 5 (25%)
ASA classification (I/II)	4 (20%) / 16 (80%)
Left / right	7 (35%) / 13 (65%)
Patella resurfacing (yes / no)	11 (55%) / 9 (45%)

MDRD = modification of diet in renal diseases
ASA = American Society of Anaesthesiologists.

ling the cumulative concentration, because the filtered shed blood would be returned to the patient.

The ropivacaine concentrations in shed blood were much higher in comparison to the plasma levels. The unbound ropivacaine fraction (mean ± SD) in shed blood (68.8 ± 4.6%) was higher as compared to plasma (4.8 ± 1.1%).

When the shed blood would have been returned to the patient an average of 13.1 ± 3.7 mg (range : 6.2-18 mg) unbound ropivacaine would have been administered intravenously.

We estimated the cumulative ropivacaine plasma concentration when the shed blood would have been returned to the patient. This model showed a mean unbound ropivacaine plasma concentration after retransfusion of the shed blood of 0.26 ± 0.11 mg/L (range : 0.12-0.58 mg/L).

Haemoglobin levels showed the expected decrease during the first postoperative days in all patients. The average pre-operative Hb value was 9.1 mmol/L (SD : 0.5 mmol/L) ; on day 1 and 3 Hb values were respectively 6.9 mmol/L (SD : 0.6 mmol/L) and 6.6 mmol/L (SD : 0.8 mmol/L). None of the patients received an allogeneic blood transfusion.

DISCUSSION

LIA is a relatively simple technique where significant opiate sparing effects have been described (*9,11*). The combination with a retransfusion drain is performed in several hospitals, however questions were raised with respect to its safety. Before addressing efficacy we need to examine the issues of safety. We combined these two modalities with special focus on the unbound ropivacaine plasma concentration, since the unbound fraction is mainly responsible for systemic toxicity. We expected that the cumulative modelled unbound ropivacaine plasma concentration at 6 hours postoperatively would be well below the threshold for systemic toxicity stated by Knudsen *et al* (*14*). They performed a study on healthy volunteers receiving ropivacaine intravenously. Based on the arterial sampling a threshold for CNS (central nervous system) toxicity is apparent at mean (min-max) unbound ropivacaine plasma concentration in the order of 0.56 mg/L (range : 0.34-0.85 mg/L) (*14*). Without retransfusion we found unbound ropivacaine plasma concentrations of 0.05 ± 0.02 mg/L (95% CI : 0.04-0.06 mg/L) at 6 hours postoperative.

It is important to base the safe limits on the

Table II. — Total and unbound ropivacaine concentrations in plasma and shed blood

	Unbound	Total	Fraction
T = 0	0.014 ± 0.009 (0.003-0.036)	0.302 ± 0.177 (0.078-0.698)	4.5 ± 0.6 (3.1-5.3)
T = 3	0.042 ± 0.019 (0.019-0.086)	0.798 ± 0.254 (0.448-1.434)	5.1 ± 0.9 (3.3-7.1)
T = 6	0.050 ± 0.022 (0.025-0.105)	0.888 ± 0.299 (0.539-1.689)	5.8 ± 2.2 (3.4-12.6)
T = 24	0.040 ± 0.015 (0.017-0.078)	1.028 ± 0.328 (0.467-1.886)	3.9 ± 0.6 (2.7-5.1)
ABT before	23.05 ± 5.72 (11.95-32.69)	33.049 ±6.539 (18.445-41.773)	69.1 ± 4.7 (62.2-78.5)
ABT after	22.61 ± 5.72 (10.33-31.87)	32.699 ±6.777 (16.385-41.300)	68.5 ± 4.5 (59.9-77.2)

Unbound and total values are shown in mg/L as mean ± SD (range).
The "fraction" is unbound concentration in % of the total concentration.
Values under timepoints (T) 0, 3, 6 and 24 hours represent plasma.
Values under "ABT" (Autologous Blood Transfusion) before and after represent shed blood before and after filtration.

unbound plasma concentrations since this concentration is related to systemic pharmacodynamic effects and toxicity. Two studies (*6,8*) used LIA intraoperatively with ropivacaine in dose up to 400 mg generating unbound ropivacaine plasma concentrations well below the threshold for systemic toxicity (*14*).

Four studies have so far been published where shed blood has been collected postoperatively and analysed for ropivacaine content (*4,10,18,21*). In these studies, the total amount of ropivacaine in the shed blood collected has found to be low, < 27 mg, in comparison to doses used in regional anaesthesia. In one of these studies (*21*) doses up to 490 mg were used. In our study we found a maximum total ropivacaine concentration in shed blood of 41.3 mg. The reason for this slightly higher value might be explained by our study design. We started the postoperative wound infusion immediately after the end of surgery in difference with all the other published studies where the wound infusion started after six hours, e.g after finalisation of drain blood collection.

No investigation has so far been published measuring the change in unbound ropivacaine following retransfusion of shed blood. However in one study the change in total ropivacaine was measured (*18*). The estimated mean maximum dose of ropivacaine reinfused, obtained from the product of drain volume and concentration, was approximately 1.3 mg (range : 0.4-2.6 mg). The mean total ropivacaine plasma concentration increased slightly, 0.03 μg/mL, from 0.79 μg/mL to a mean value of 0.82 μg/mL after completion of retransfusion (*18*). In our study the total amount of ropivacaine in shed blood was 32.7 mg (range : 16.4-41.3 mg). Considering a worst case scenario where shed blood with 41.3 mg ropivacaine would be reinfused our values would be 32 times (41.3/1.3) higher than by Parker *et al* (*18*). Theoretically this would with our study results generate a blood level increase of total ropivacaine with 32×0.03 mg = 0.95 mg/L, when the shed blood had been reinfused.

The major determinant of systemic toxicity in local anaesthetics is the unbound concentration in plasma. It is known that AAG, the protein responsible for ropivacaine binding, has high inter- and intra-individual variability and therefore concentrations and binding capacity can largely differ over time and between patients. Furthermore, AAG concentrations are influenced by surgery, myocardial infarction and inflammatory processes (*24*). Essving *et al* noted that even though the total plasma concentration showed increasing values, the free fraction decreases with time (*8*). This is in line with the fact that ropivacaine is mainly bound to AAG. The AAG availability has been associated with an increase in the protein binding of ropivacaine during long-term infusion after surgery (*5,23*). This is also seen in our study were the mean unbound ropivacaine fraction decreases after 24 hours. In our study mean unbound ropivacaine fraction was 4.8% in the included ASA I and II patients. This is lower than in Knudsen's study which may be explained by the fact that our patients had more co-morbidity (*14*).

In two previously published studies the unbound ropivacaine fraction was measured (*4,8*). The unbound ropivacaine fraction in the different studies varied between 2.2 and 8.8%. In our study we found unbound ropivacaine fraction in plasma at six hours to be 5.8% (range, 3.4-12.6%). Applying the highest fraction on the theoretically generated total plasma level increase of 0.95 mg/L would generate unbound ropivacaine, if reinfused, of $12.6\% \times 0.95$ mg/L = 0.12 mg/L. Adding this maximum calculated increase to the highest reported unbound ropivacaine plasma concentration generates a maximum unbound ropivacaine plasma concentration of 0.105 + 0.12 = 0.225 mg/L, a concentration well below the threshold for systemic toxicity of 0.56 mg/L (*14*). Also the model we used with all specific data from each patient (e.g. plasma volume, shed blood volume and free fraction) showed a mean unbound ropivacaine plasma concentration after retransfusion of the shed blood of 0.26 ± 0.11 mg/L (range, 0.12-0.58 mg/L). In both models we assume instant retransfusion and instant binding. In daily practice however, an erythrocyte concentrate is normally reinfused in half an hour or more, so even lower unbound ropivacaine values are likely.

Regarding the shed blood collected, the median total drainage loss of 600 mL was similar to the reported amounts in the study of Vendittoli *et*

al (*22*). Vendittoli noted that drainage loss was not significantly different between patients with single morphine consumption or in combination with periarticular infiltration.

In conclusion, data so far indicate that intraoperative local infiltration analgesia with ropivacaine for hip and knee arthroplasty can safely be combined with autologous blood reinfusion, even if a postoperative ropivacaine wound infusion at low rates starts directly after the end of surgery. Nonetheless, the safety issues have to be warranted by actual administration of the shed blood collected.

REFERENCES

1. **Abuzakuk T, Senthil K, V, Shenava Y *et al.*** Autotransfusion drains in total knee replacement. Are they alternatives to homologous transfusion ? *Int Orthop* 2007 ; 31 : 235-239.
2. **Amin A, Watson A, Mangwani J *et al.*** A prospective randomised controlled trial of autologous retransfusion in total knee replacement. *J Bone Joint Surg* 2008 ; 90-B : 451-454.
3. **Bianconi M, Ferraro L, Traina GC *et al.*** Pharmacokinetics and efficacy of ropivacaine continuous wound instillation after joint replacement surgery. *Br J Anaesth* 2003 ; 91 : 830-835.
4. **Breindahl T, Simonsen O, Andreasen K.** Column-switching HPLC-MS/MS analysis of ropivacaine in serum, ultrafiltrate and drainage blood for validating the safety of blood reinfusion. *J Chromatogr B Analyt Technol Biomed Life Sci* 2010 ; 878 : 76-82.
5. **Burm AG, Stienstra R, Brouwer RP, Emanuelsson BM, van Kleef JW.** Epidural infusion of ropivacaine for postoperative analgesia after major orthopedic surgery : pharmacokinetic evaluation. *Anesthesiology* 2000 ; 93 : 395-403.
6. **Busch CA, Shore BJ, Bhandari R *et al.*** Efficacy of periarticular multimodal drug injection in total knee arthroplasty. A randomized trial. *J Bone Joint Surg* 2006 ; 88-A : 959-963.
7. **Cheng SC, Hung TS, Tse PY.** Investigation of the use of drained blood reinfusion after total knee arthroplasty : a prospective randomised controlled study. *J Orthop Surg (Hong Kong)* 2005 ; 13 : 120-124.
8. **Essving P, Axelsson K, Gupta A, Lundin A.** Plasma concentration of ropivacaine following peri- and intraarticular injection during total knee arthroplasty. 2010. European Society of Regional Anaesthesia Congress. Unpublished Work.
9. **Essving P, Axelsson K, Kjellberg J *et al.*** Reduced hospital stay, morphine consumption, and pain intensity with local infiltration analgesia after unicompartmental knee arthroplasty. *Acta Orthop* 2009 ; 80 : 213-219.
10. **Gill I, Gallagher K, Busch CA.** Is peri-articular multimodal drug infiltration in knee arthroplasty safe when used in conjunction with autologous retransfusion drains ? *Ann R Coll Surg Engl* 2010 ; 92 : 335-337.
11. **Gomez-Cardero P, Rodriguez-Merchan EC.** Postoperative analgesia in TKA : ropivacaine continuous intraarticular infusion. *Clin Orthop Relat Res* 2010 ; 468 : 1242-1247.
12. **Graf BM, Abraham I, Eberbach N *et al.*** Differences in cardiotoxicity of bupivacaine and ropivacaine are the result of physicochemical and stereoselective properties. *Anesthesiology* 2002 ; 96 : 1427-1434.
13. **Kerr DR, Kohan L.** Local infiltration analgesia : a technique for the control of acute postoperative pain following knee and hip surgery : a case study of 325 patients. *Acta Orthop* 2008 ; 79 : 174-183.
14. **Knudsen K, Beckman SM, Blomberg S, Sjovall J, Edvardsson N.** Central nervous and cardiovascular effects of i.v. infusions of ropivacaine, bupivacaine and placebo in volunteers. *Br J Anaesth* 1997 ; 78 : 507-514.
15. **Lemmens HJ, Bernstein DP, Brodsky JB.** Estimating blood volume in obese and morbidly obese patients. *Obes Surg* 2006 ; 16 : 773-776.
16. **Moonen AFCM.** *Postoperative Autologous Retransfusion of shed blood in Primary Total Hip and Knee Arthroplasty*. Dissertation 5-12-2008, University of Maastricht.
17. **Otte KS, Husted H, Andersen LO, Kristensen BB, Kehlet H.** Local infiltration analgesia in total knee arthroplasty and hip resurfacing : A methodological study. *Acute Pain* 2008 ; 10 : 111-116.
18. **Parker DA, Coolican MR, Mather LE, Graham DA, Dewall MJ.** Safety of combined use of local anesthetic infiltration and reinfusion drains in total knee arthroplasty. *J Arthroplasty* 2009 ; 24 : 918-924.
19. **Parker MJ, Roberts CP, Hay D.** Closed suction drainage for hip and knee arthroplasty. A meta-analysis. *J Bone Joint Surg* 2004 ; 86-A : 1146-1152.
20. **Strassels SA, Chen C, Carr DB.** Postoperative analgesia : economics, resource use, and patient satisfaction in an urban teaching hospital. *Anesth Analg* 2002 ; 94 : 130-7, table.
21. **Stringer BW, Singhania AK, Sudhakar JE, Brink RB.** Serum and wound drain ropivacaine concentrations after wound infiltration in joint arthroplasty. *J Arthroplasty* 2007 ; 22 : 884-892.
22. **Vendittoli PA, Makinen P, Drolet P *et al.*** A multimodal analgesia protocol for total knee arthroplasty. A randomized, controlled study. *J Bone Joint Surg* 2006 ; 88-A : 282-289.
23. **Wiedemann D, Muhlnickel B, Staroske E, Neumann W, Rose W.** Ropivacaine plasma concentrations during 120-hour epidural infusion. *Br J Anaesth* 2000 ; 85 : 830-835.
24. **Yost RL, DeVane CL.** Diurnal variation of alpha 1-acid glycoprotein concentration in normal volunteers. *J Pharm Sci* 1985 ; 74 : 777-779.
25. **Zink W, Graf BM.** The toxicity of local anesthetics : the place of ropivacaine and levobupivacaine. *Curr Opin Anaesthesiol* 2008 ; 21 : 645-650.