A randomized controlled trial comparing haemodynamic stability in elderly patients undergoing spinal anaesthesia at L5, S1 versus spinal anaesthesia at L3, 4 at a tertiary African hospital.

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Abstract
Background: Spinal anaesthesia is a routinely used anaesthetic technique in elderly patients (> 60 years) undergoing operations involving the lower limbs, lower abdomen, pelvis and the perineum. Spinal anaesthesia has several advantages over general anaesthesia including stable haemodynamic variables, less blood loss, less post-operative pain, faster recovery time and less post-operative confusion. Despite these advantages, the sympathetic blockade induced by spinal anaesthesia can result in hypotension, bradycardia, dysrhythmias and cardiac arrests. Conventionally, spinal anaesthesia is performed at the level of L3,4 interspace; with a reported incidence of hypotension in the elderly ranging between 65% and 69%. A possible strategy for reducing spinal induced hypotension would be to minimize the peak block height to as low as possible for the planned procedure.

Objective: To determine the difference in haemodynamic stability between elderly patients undergoing spinal anaesthesia at L5, S1 interspace compared to those at L3, 4.

Methods: Thirty two elderly patients scheduled for lower limb or pelvic surgery under spinal anaesthesia were randomized into 2 groups (control group and intervention group) using a computer generated table of numbers.

Control group; received 2.5 mls 0.5% hyperbaric bupivacaine injected intrathecally at the L3, 4 interspace and Intervention group; 2.5mls 0.5% hyperbaric bupivacaine injected intrathecally at the L5, S1 interspace

Results: The two groups had similar baseline characteristics in age, sex, body mass index and use of anti-hypertensive medications. There was 68.8% proportion of hypotension in the control group and 75% in the intervention group. The difference was not found to be statistically significant (p= 0.694). During the study period, there were 106 episodes of hypotension, out of which, 65 were in the control group and 41 in the intervention group (p=0.004). Linear regression analysis of the decrease in mean arterial pressures (MAP) showed a higher decrease in MAP in the control group (p = 0.018). There were more crystalloids used in the control group (1006mls ± 374) than in the intervention group (606mls ±211) with a p< 0.0001. There was no difference in the amounts of vasopressors used between the two groups (p=0.288). There was no difference in the change in heart rates, conversion to general anaesthesia, use of supplementary intravenous fentanyl and the peak maximum block level achieved. The time to peak maximum sensory block level was 9.06min and 13.07min in the control group and intervention groups, respectively (p<0.0001).

Conclusion: Among this population, there was no difference in the proportion of those with hypotension between the elderly patients who received their spinal anaesthesia at L3,4 and those who received spinal anaesthesia at L5,S1. The intervention group had better outcomes with significantly less episodes of hypotension. It took a longer time to achieve a maximum peak sensory block in the intervention group. Performing spinal anaesthesia at the level of L5,S1 was found to provide an adequate sensory block for a wide range of pelvic, perineal and lower limb surgeries.

Keywords: Haemodynamic stability, spinal anaesthesia

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Introduction

Spinal anaesthesia (SA) consists of the temporary interruption of nerve transmission within the subarachnoid space produced by injection of a local anesthetic solution into cerebrospinal fluid (CSF). SA is a routinely used anaesthetic technique for operations involving the lower limbs, lower abdomen, pelvic and perineal surgeries1,3. An increasing proportion of the patients undergoing these surgical procedures are the elderly. Age related changes in physiology and pharmacology can affect every aspect of peri-operative care4.

The use of spinal anaesthesia is increasing in popularity compared to general anaesthecia5–7. Spinal anaesthesia has many potential advantages over general anaesthesia (GA) between the two groups and to determine the level of sensory block in patients undergoing spinal anaesthesia at the level of L5, S1.

Methodology

The study was performed following approval from the ethical and scientific research committee at the Aga Khan University, East Africa.

Patients were recruited after having signed an informed consent, which clearly stated that this was a study being conducted and that their personal information would be kept confidential. They were informed and consented to the study. They further consented on the findings being published.

This was a randomized single blinded controlled trial. The study was conducted at the Aga Khan University Hospital, Nairobi. The Aga Khan university Hospital, Nairobi (AKUHN) is a 254 bed private-not-for-profit institution that provides tertiary and secondary level health care services. The hospital serves the residents of Nairobi and also receives referrals from other parts of the country and the continent. It is a teaching hospital that offers courses in postgraduate medical education and advanced nursing. It has five operating theatres with approximately 8,000 surgical procedures performed in 2011.

The target population included all elderly patients, aged 60 years and above, admitted for lower limb and pelvic surgeries at the Aga Khan University hospital operating theatres. The sample population included all elderly American Society of Anesthesiologists (ASA) physical status I to III patients scheduled for surgical procedures that were amenable for spinal anaesthesia (lower limb and pelvic surgeries) in the period between October 2011 and March 2012. All elderly ASA I –III patients scheduled to undergo lower limb and pelvic surgeries were included in this study.

Reasons for exclusion from the study were:
1. Patient refusal to participate in the study
2. Contraindication to spinal anaesthesia
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Simple randomization was used. Using a computer program, the principal investigator generated a random sequence of numbers. Each of the random numbers was sequentially assigned to either; Control group; 2.5 mls 0.5% hyperbaric bupivacaine injected intrathecally at the L3, 4 interspace. Intervention group: 2.5 mls 0.5% hyperbaric bupivacaine injected intrathecally at the L5, S1 interspace.

The study was undertaken at the Aga Khan University Hospital Nairobi operating theatres. 32 elderly (ASA physical status I–III) patients scheduled for lower limb surgery were randomized to receive 2.5 mls of 0.5% hyperbaric bupivacaine intrathecally at the L3, 4 interspace (control group) or at the L5, S1 interspace (intervention group).

On arrival in the operating theatre, standard monitoring was applied with automated noninvasive blood pressure measurement, electrocardiography and pulse oximetry. Baseline mean arterial blood pressure (MAP) and heart rate (HR) were recorded while lying down comfortably and the average of 3 readings was taken as the baseline blood pressure. Subsequently, the blood pressure was measured at 2.5 min intervals in the position of surgery. All patients received 500mls of lactated Ringer's solution during induction of the allocated spinal anesthetic technique to run over the first 30 minutes. The patient was then positioned in a sitting position. After cleaning and draping, the allocated interspace was identified by palpation then confirmed with the assistance of an imaging intensifier. An imaging intensifier, which emits very low radiation dose, was used to determine the interspaces. Imaging was kept at a minimum and patients did not undergo any more radiation exposure than would be normally required for the confirmation of the intervertebral space. All the staff involved wore protective shielding with lead aprons and thyroid shields to prevent exposure to scatter radiation during use of the imaging intensifier.

5 mls of 2% plain lignocaine was then infiltrated on the skin. The spinal anesthesia was performed with the patient in the sitting position using a midline approach at the L3, 4 interspace for the standard group; and the L5, S1 interspace for the low block group. A 22 or 25 gauge spinal needle was used and after CSF flow was obtained, 2.5 mls of hyperbaric bupivacaine was injected over 10 seconds with barbotage. The patient was then turned supine and left supine for 10 minutes. Five minutes from completion of the intrathecal injection (taken as the point of removal of the spinal needle), the sensory block level to both light touch and cold were checked at 2.5 min intervals until there was no change in 3 consecutive readings. To assess the level of block to light touch, a dry cotton wool swab was used; and for loss of cold sensation, cold ethyl chloride spray was used.

Surgery was allowed to commence as soon as the sensory block height to light touch had been tested pre-inception and reached the tenth thoracic dermatome (T10).

The operation did not start until it was confirmed by testing pre-inception that the anesthesia was adequate for the procedure.

In case of any discomfort or pain, we used IV paracetamol 1gm and IV fentanyl 1-2 mcg/kg and the patient was offered general anesthesia (GA).

Hypotension (defined as a reduction in MAP of more than 20% from baseline determined just before the administration of spinal anesthesia or MAP below 60mmHg) was treated with ringer’s lactate 200mls bolus, ephedrine boluses of 6 mg to a total of 30 mg and consequently fluids titrated to effect on the blood pressures. If this was not enough to return the blood pressures to a MAP above 60mmHg, phenylephrine boluses 50mcg titrated to effect were used.

Bradycardia (defined as a heart rate below 60 beats per minute) was treated with atropine 0.3mg to 0.6mg titrated to effect.

The presence of intraoperative nausea, vomiting, pruritus, and shivering was noted and treated appropriately. Rescue antiemetic drugs using a combination of IV ondansetron 4mg and dexamethasone 8mg were administered. Discomfort from post anaesthetic shivering was treated with IV pethidine 25mg.

Post-operative analgesia was prescribed at the discretion of the primary anaesthesiologist attending to the patient.

The patient’s bio data, medical history and level of spinal injection used relevant to the study were recorded by the anaesthesiologist who performed the SA. Intraoperative data was collected by the principal investigator or a trained research assistant after SA had been performed using a data collection form.
All the raw data in this study was filed in a suitable box file which was stored in a lockable filing drawer. All data was verified for completion by the principal investigator before being filed. Every precaution was taken to respect the privacy of patients whose data was collected and analyzed in this study. Patient data was only identified by a unique identifier number. In the course of monitoring data quality and adherence to the study protocol only the study supervisors could refer to recruited patients’ medical records.

Data analysis was undertaken using the STATA/SE 11 (from StrataCorp USA) with the input of a statistician who has been involved since the beginning of the study.

Descriptive statistics were used to compare patients’ characteristics in terms of age, sex, height, weight, baseline blood pressures and heart rates. Student’s T test was used to compare the differences between blood pressure reduction and heart rates reduction between the two groups.

The Chi test was used to compare the proportions of episodes of hypotension in the 2 groups. The statistician offered guidance during data entry, analysis and presentation of the final statistics.

Results
Thirty two elderly (aged above 60 years) patients who underwent spinal anaesthesia were included in this study. Their baseline characteristics are shown in table 1.

The 2 groups were similar with no significant difference in their baseline characteristics. The mean age was 66 years in the control group and 69 years in the intervention group. The body mass index (BMI) in the control arm was 29.22 versus 27.27 in the intervention arm but this difference was not statistically significant. There were more men in both groups of the study. 81.25% of the patients in the control group had chronic illnesses compared to 87% in the intervention group while in both groups 50% of the patients were on anti-hypertensive medication.

The differences between the two groups in total fluids given and total ephedrine and phenylephrine used were compared using Mann-Whitney non parametric statistical test.

Maximum sensory block achieved was analyzed using the Mann-Whitney test.

Survival time analysis (Kaplan Meir) was used to analyze the time to hypotension. Log rank test was used to compare the rate of hypotension in the 2 groups. The results on the secondary outcomes-change in heart rate, the use of vasopressors and the level and time of onset of maximum blocks between the control (L3,4) and the intervention (L5,S1) groups, have been shown on tables 5 and 6.

The percentage of patients converted to general anaesthesia in both groups was analyzed using the Z test for equality of proportions.

Further, in table 3, the results show the number of episodes of hypotension recorded during the period of the study(45 minutes), followed by some descriptive statistics and graphs on the same.

### TABLE 1: Patients’ baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Intervention</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>65.75(4.64)</td>
<td>68.75(8.72)</td>
<td>0.883</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.625(10.81)</td>
<td>76.19(19.66)</td>
<td>0.400</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.94(6.84)</td>
<td>166.25(12.47)</td>
<td>0.820</td>
</tr>
<tr>
<td>BMI</td>
<td>29.22(3.581)</td>
<td>27.27(5.09)</td>
<td>0.110</td>
</tr>
<tr>
<td>Sex: Female (Male)</td>
<td>7 (9)</td>
<td>5 (11)</td>
<td>0.465</td>
</tr>
<tr>
<td>Chronic Illness</td>
<td>13</td>
<td>14</td>
<td>0.626</td>
</tr>
<tr>
<td>Anti-hypertensives use</td>
<td>8</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Other drugs</td>
<td>11</td>
<td>8</td>
<td>0.28</td>
</tr>
</tbody>
</table>

The results on the secondary outcomes-change in heart rate, the use of vasopressors and the level and time of onset of maximum blocks between the control (L3,4) and the intervention (L5,S1) groups, have been shown on tables 5 and 6.

### Table 5 Secondary outcomes

<table>
<thead>
<tr>
<th></th>
<th>Control(SD)</th>
<th>Intervention(SD)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradycardia</td>
<td>10%</td>
<td>15%</td>
<td>0.132</td>
</tr>
<tr>
<td>Fluids used ml (SD)</td>
<td>1006(374)</td>
<td>606 (211)</td>
<td>0.001</td>
</tr>
<tr>
<td>Ephedrine used, in mg(SD)</td>
<td>15 (10.8)</td>
<td>8.4 (7.1)</td>
<td>0.288</td>
</tr>
<tr>
<td>Ephedrine used (% patients)</td>
<td>37.5%</td>
<td>31.25%</td>
<td>0.710</td>
</tr>
<tr>
<td>Converted to GA n (%)</td>
<td>1(6.25%)</td>
<td>2(12.5%)</td>
<td>0.544</td>
</tr>
<tr>
<td>Supplementary analgesia (I.V Fentanyl) n (%)</td>
<td>2 (12.5%)</td>
<td>1 (6.25%)</td>
<td>0.544</td>
</tr>
<tr>
<td>Time ,in minutes, to maximum block(SD)</td>
<td>9.06(5.2)</td>
<td>13.07(7.9)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

GA-General anaesthesia, I.V –intravenous, SD –Standard deviation

### TABLE 6 Level of maximum sensory block

<table>
<thead>
<tr>
<th></th>
<th>Intervention</th>
<th>Control</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensory block</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block to light touch</td>
<td>T.9(2.0)</td>
<td>T.8(2.0)</td>
<td>0.08</td>
</tr>
<tr>
<td>Block to cold</td>
<td>T.10.1(1.6)</td>
<td>T.9.1(2.1)</td>
<td>0.054</td>
</tr>
</tbody>
</table>

#### TABLE 2 Proportion of hypotension (primary outcome)

<table>
<thead>
<tr>
<th></th>
<th>Control (L3,4)</th>
<th>Intervention (L5,S1)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No hypertension n(%)</td>
<td>5 (31.3%)</td>
<td>4 (25%)</td>
<td></td>
</tr>
<tr>
<td>Hypotension n(%)</td>
<td>11(68.8%)</td>
<td>12(75%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>16(100%)</td>
<td>16(100%)</td>
<td></td>
</tr>
</tbody>
</table>

P Value 0.694

#### TABLE 3 Episodes of hypotension during the first 45 minutes of SA

<table>
<thead>
<tr>
<th></th>
<th>Control (L3,4)</th>
<th>Intervention (L5,S1)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No hypotension n (%)</td>
<td>95 (59.38%)</td>
<td>119 (74.38%)</td>
<td>214(66.88%)</td>
</tr>
<tr>
<td>Hypotension episodes n (%)</td>
<td>65 (40.63%)</td>
<td>41 (25.62%)</td>
<td>106 (33.13%)</td>
</tr>
<tr>
<td>Total n (%)</td>
<td>160 (100%)</td>
<td>160 (100%)</td>
<td>320 (100%)</td>
</tr>
</tbody>
</table>

Pearson chi test 8.1256

P Value 0.004
The data was analysed to verify statistical significance, which was defined as p value less than 0.05. Figure 2 shows the number of episodes of hypotension in the first forty-five minutes of the spinal anaesthesia.

Figure 2 Number of episodes of hypotension per patient during the first 45 minutes of spinal anaesthesia

There was 68.75% incidence of at least one episode of hypotension in the control group (L3,4) and 75% in the intervention group. This was not found significant (p value of 0.694). There were 10 blood pressure readings for each patient during the first forty-five minutes of the spinal anaesthesia (at 2.5 min, 5 min, 7.5 min, 10 min, 12.5 min, 15 min, 20 min, 25 min, 30 min and 45 min), giving a total of 320 readings. 106 out of these 320 readings were hypotensive pressures. The control group had 65/106 while the intervention group had 41/106 hypotensive episodes. There was a significant difference in the number of hypotensive episodes between the two groups (p value 0.004).

Figures 3 and 4 illustrate the mean arterial pressures change over time.

Figure 3 Mean Arterial Pressures (MAP) over time

A linear regression analysis shown in table 4 revealed a statistically significant difference between change in mean arterial pressures (MAP) in the control group (L3,4) and the intervention group (L5,51).

TABLE 4 Linear regression analysis comparing control versus intervention for Mean Arterial Pressure (MAP) change

A linear regression analysis revealed a statistically significant difference between change in mean arterial pressures (MAP) in the control group (L3,4) and the intervention group (L5,51).

Figure 6 illustrates heart rate changes over time in the two groups.

Figure 4 Change in Mean arterial pressure (MAP) over time
The Kaplan Meir curves in figure 5 demonstrate that time to onset of hypotension was most likely to occur between ten and thirty minutes in both groups; with the control group having more episodes of hypotension compared to the intervention.

There was a significant difference in the amount of intravenous fluids (Ringer's Lactate) used between the two groups (p= 0.001); but not in the amount of vaso-pressors used in the patients as shown in table 5. There was no difference in the number of patients converted to general anaesthesia or those who required supplementary intravenous fentanyl. The difference in the time to maximum sensory block achieved was found to be significant (p=0.0001), being longer in the intervention arm.

There was no statistically significant difference in the peak block heights to both cold and light touch between the two groups (table 6, figure 7 and 8).
In summary, these results show that there was a significant difference in the number of hypotensive episodes between the two groups, and no difference in the proportion (of at least one episode) of hypotension. The change in heart rate, the use of vasopressors, and the rate of conversion to general anaesthesia were not found to be different between the two groups. The difference in the maximum sensory block level achieved was not significant but the difference in the time of onset of maximum blocks achieved was significant.

Discussion

The principal finding of this study was that the total numbers of episodes of hypotension were significantly less in the intervention group (L5, S1) compared to the control group (L3,4). This difference was statistically significant (p value 0.004). However, the proportion of hypotension in the two was not statistically significant. In this study, we defined hypotension as a 20% decrease in mean arterial pressure (MAP) from baseline or MAP of below 60mmHg. There is a paucity of published well designed studies on spinal anaesthesia at the level of L5, S1. The published case reports, of one patient each, showed haemodynamic stability in the participants.

In contrast to our hypothesis, the proportion of hypotension was higher in the intervention group than in the control group.

The findings in the control group correspond to the published incidence of hypotension of 65% to 69%[12]. The difference in the proportions of patients who had hypotension in the control and in the intervention groups was not statistically significant. This shows that performing spinal anaesthesia at L5,S1 does not reduce the proportion of hypotension, thus disproving our hypothesis. These findings clearly show that although the proportion of patients who had hypotension was not reduced by performing spinal anaesthesia at the lower level of L5,S1 as hypothesized, the number of episodes of hypotension were significantly reduced making them more haemodynamically stable than those patients who had spinal anaesthesia performed at L3,4.

In the current study, bradycardia was defined as a heart rate below 60 beats per minute. The prevalence of bradycardia in the control group compared to the intervention group was found to be statistically insignificant. These findings are similar to those of Carpenter et al who reported a 13% incidence of bradycardia[1]. None of the patients required rescue atropine for the bradycardia as it either resolved spontaneously or responded to rescue epinephrine doses as the bradycardia was associated with hypotension. In this study, we did not record any other dysrhythmias on ECG, and none of our patients required cardiopulmonary resuscitation.

There was a statistically significant difference between the two groups in the amount of intravenous fluids (Ringer’s Lactate) used but no difference in the amounts of epinephrine used (table 5). This probably reflects the difference in the number of episodes of hypotension between the two groups as there were more episodes in the control group (L3,4) compared to the intervention (L5,S1) group. As per the study protocol, whenever hypotension was noted, a bolus of intravenous fluid was administered before administering a vasopressor. This also reflects the practice in the study hospital, where the anaesthesiologist administers a crystalloid bolus in case of a decrease in blood pressures, and if there’s no response, vasopressors (epinephrine) boluses are added. None of the patients received phenylephrine.

During this study one patient (6.25%) in the control group was converted to general anaesthesia in the control group and two patients (12.5%) in the intervention group (p value 0.544). The reason was that in all the 3 patients, there was an inadequate sensory block for the procedures.

The differences in the peak sensory block both to cold and touch, between the two groups, were not found to be statistically significant (table 6). These findings correspond to those of Veering et al who did not find any difference in maximum level of analgesia when comparing spinal anaesthesia at L3,4 and L4,5 in elderly patients[16]. During the study, the mean time to maximum block was 9 minutes and 13 minutes for the control group and intervention group, respectively (table 3). This difference was statistically significant but it was not found to be clinically significant as the cases were dealt with were not being performed as emergency cases. Previous published studies on spinal anaesthesia in elderly patients report a mean time to maximum onset of block as 15 minutes with a range of 11 to 20 minutes[13,14]. This difference in time to maximal block was probably because our study tested for loss of sensation 5 minutes from completion of spinal anaesthesia then every 2.5 minutes interval until there was no change in 3 consecutive readings; while these previous studies tested for loss of sensation until 30 minutes after spinal anaesthesia[13,14].

As it is well known that lumbar spaces may be misidentified by use of clinical palpation alone, in this study an X ray image intensifier was used to overcome this technical challenge of accurately identifying the interspaces in all the patients. Previous studies have found that clinical palpation of the lumbar interspaces were only 30 % accurate[27,28,29].

Performing spinal anaesthesia at the level of L5, S1 was found to provide an adequate block for a wide range of urological procedures (TURP, bladder neck incision, orchidopexies), orthopaedic procedures on the lower limbs, gynaecologic (hysterectomies, vaginal fistula repair) and general surgical procedures like inguinal herniorrhaphy (Table 2). Peak sensory block, use of supplementary analgesia (intravenous fentanyl) and the rate of conversion to general anaesthesia were used as indicators for adequacy of block achieved for the surgical procedures performed. These differences were found to be statistically significant. The rate of conversion to general anaesthesia and the use of intravenous fentanyl in the intervention group were also not significantly different from the control group (table 5).

Although performing spinal anaesthesia at the lower level of L5,S1 (compared to the conventional level of L3,4) does not eliminate the occurrence of hypotension, there are significantly less hypotensive episodes per patient with no difference in heart rate changes and a similar peak sensory block. In view of these findings, we concluded that in elderly patients, a spinal anaesthetic at L5,S1 results in a more haemodynamically stable patient, with a sufficient sensory blockade achieved, thus making it a safer level for performing spinal anaesthesia.

Strengths of the study

After a rigorous literature review, it appears that this is the first prospective randomized controlled study on performing spinal anaesthesia at the level of L5,S1. Therefore, this study will add to the scarce body of literature and knowledge on spinal anaesthesia performed at the level of L5,S1 and probably form a basis for many other studies on spinal anaesthesia in the future. In the current study, fluoroscopy was used to confirm the spinal interspace used for spinal anaesthesia. This gives 100% accuracy in the identification of the spinal interspaces used for the study.

Limitations of the study

The study was conducted at a single centre and involved a relatively small number of patients and a wide range of procedures. This may impact on the generalizability of the results of this study. Three patients in the study were converted to general anaesthesia and this could have confounded our results.

Conclusion

On the basis of the results of this study, there was no difference between the proportion of hypotension in elderly patients undergoing spinal anaesthesia at the level of L5,S1 and those undergoing spinal anaesthesia at the level of L3,4. However, the number of hypotensive episodes were significantly more in the control group (L3, 4) than in the intervention group (L5,S1). This difference was statistically significant. The difference in heart rate change (bradycardia) between the two groups was also not statistically significant. Therefore, on the basis of this study, we conclude that performing spinal anaesthesia at the level of L5, S1 in the elderly patient was found to provide an adequate block for a wide range of urological procedures (TURP, Bladder neck incision, orchidopexies), orthopaedic procedures on the lower limbs, gynaecologic (hysterectomies, vaginal fistula repair) and general surgical procedures like inguinal herniorrhaphies.

The study was registered under Pan African Clinical Trials Registration number PACTR 201100000311318.