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ORIGINAL ARTICLE

# Gender differences in degenerative spine surgery: Do female patients really fare worse?

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## Abstract

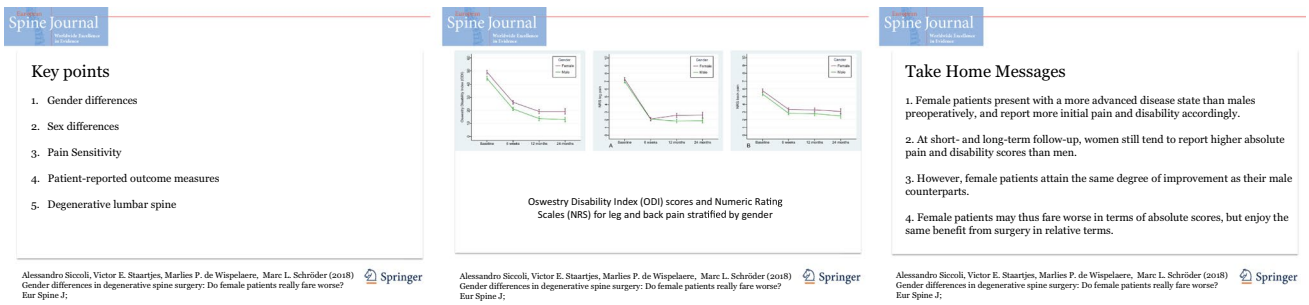
**Purpose** Prior data has set the precedent that female patients fare somewhat worse than men after spine surgery. We aimed to evaluate the effect of gender on patient-reported outcomes after lumbar spine surgery for degenerative pathologies.

**Methods** We identified a consecutive cohort of patients from a prospective registry. Absolute values, as well as change scores for back and leg pain severity (numeric rating scale [NRS]), functional disability (Oswestry disability index [ODI]), and health-related quality of life (HRQOL) as assessed by EQ-5D were compared among male and female patients.

**Results** Of the 3279 included patients, 1543 (47%) were female. At baseline, women reported higher NRS for back and leg pain, higher ODI, but equal HRQOL (all $p < 0.05$). Otherwise, both groups had comparable baseline data. The absolute differences in patient-reported outcomes persisted at the 6-week, 12- and 24-months follow-up, with women now additionally reporting worse HRQOL as assessed by EQ-5D (all $p < 0.05$). For all outcome measures, change scores were equal among male and female patients, as were the incidences of complications and reoperations (all $p > 0.05$). Clinical success was achieved in 82% of men and 79% of women ($p = 0.34$).

**Conclusions** Female patients are generally scheduled for surgery with a more advanced disease state. While women seem to report more severe symptoms at long-term follow-up, the degree of improvement is equal among men and women. Female patients may thus fare worse in terms of absolute scores, but enjoy the same benefit from surgery in relative terms.

**Graphical abstract** These slides can be retrieved under Electronic Supplementary Material.




**Keywords** Degenerative spine · Outcomes · Gender differences · Sex differences · Pain perception

## Introduction

In recent years, the study of gender differences has attracted massive interest in various specialties [1]. While some of the findings can be explained by biological variation among genders, the literature on the effect of gender on pain response and postoperative recovery is not consistent

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[2, 3]. For instance, women are reported to exhibit a lower pain threshold, higher pain perception, and increased inflammatory response compared to men, as measured by various methods [4–7]. For both physician and patient, it is useful to be informed about predictive properties of various demographic and surgical parameters. This information could be of prognostic value and play a vital role in shared decision-making. Clinically relevant inter-gender differences in patient-reported outcome measures (PROMs) and complications have been observed [8–11].

Further, large studies specifically examining gender differences in spinal surgery have been called for [11]. In addition, the majority of existing reports are restricted by analysing only one single outcome measure, examining only one specific surgical procedure, or are hampered by insufficient sample size and statistical power to detect gender-related differences. Previous analyses have established that women may fare worse after lumbar spine surgery for degenerative disease [8, 9, 12, 13]. However, this notion has recently been challenged by more powerful cohort studies [11, 14–19]. These recent studies suggest that differences may not be measurable, particularly when setting thresholds for clinical relevance. Still, due to the conflicting data in the peer-reviewed literature, it remains unclear whether there are any clinically relevant differences in PROMs among genders after these procedures.

In this analysis of a prospective registry, we aimed to elucidate the extent of inter-gender differences in terms of patient-reported outcome measures (PROMs), with a specific focus on baseline severity of disease and degree of improvement, as well as adverse events after lumbar spine surgery for degenerative diseases.

## Methods

### Patient population

From a prospective institutional registry of spinal interventions, all patients who underwent lumbar spine surgery for degenerative diseases were identified. Patients were operated between December 2010 and January 2018 by two senior neurosurgeons at a specialized spine centre. The patients underwent tubular microdiscectomy (tMD), mini-open laminectomy, or minimally invasive spinal fusion as described previously [17, 20]. We followed the STROBE statement when compiling this paper. All individual patients in this study provided written informed consent. The prospective registry was approved by the local institutional review board (Medical Research Ethics Committees United, Registration Number: W16.065), and this study was performed according to the Declaration of Helsinki.

### Data collection

We employed a standardized questionnaire containing a numeric rating scale (NRS) for back pain and leg pain severity and validated Dutch versions of the Oswestry Disability Index (ODI) as a measure of functional disability and the EQ-5D-3L questionnaire to capture health-related quality of life (HRQOL) [21, 22]. The EQ-5D-3L questionnaire contains the EQ-5D index and EQ visual analogue scale (EQ-VAS) and was evaluated according to the validated Dutch tariff [22]. Patients filled in questionnaires using a validated web-based tool before the first visit, and at 6 weeks, 12 and 24 months after surgery [23]. We defined ODI at 12 months as the primary endpoint. Measures of HRQOL were not routinely administered at the 24-month follow-up. All complications were systematically collected in a separate database, and reoperations were tracked.

### Statistical analysis

Continuous data are given as mean ± standard deviation, and categorical data as numbers (percentages). Clinical success was defined as reaching the minimal clinically important difference (MCID), namely $a \geq 30\%$ improvement, in ODI from baseline to the 12-month follow-up, according to the threshold proposed by Ostelo et al. [24]. We assessed change scores, defined as the degree of improvement from baseline, as well as the absolute differences between groups. Welch's two-sample *t* tests and Chi-square tests were used to determine intergroup differences. Whenever appropriate, we applied a continuity correction. We performed a power analysis to demonstrate MCID for our primary endpoint at an $\alpha = 0.05$. All analyses were carried out in R version 3.5.0 (The R Foundation for Statistical Computing, Vienna, Austria). A $p \leq 0.05$ on a two-tailed test was considered statistically significant.

## Results

Out of 3279 patients (Fig. 1), 1736 (53%) were male and 1543 (47%) were female. Baseline demographic data are provided in Table 1. Our post hoc power analysis revealed that, to detect MCID in our primary endpoint at 12 months, our sample size resulted in a statistical power of 99.7% [18].

Perioperative and surgical parameters are given in Table 2. Surgical time (Delta (Δ) 3.3 min., 95% confidence interval (CI) 0.52–6.1 min., $p = 0.02$) and length of stay (Δ 0.101 d., 95% CI 0.059–0.141 d., $p < 0.001$) were prolonged in female patients. Estimated blood loss was greater in male patients (Δ 33.6 mL, 95% CI 16.9–50.2 mL, $p < 0.001$).

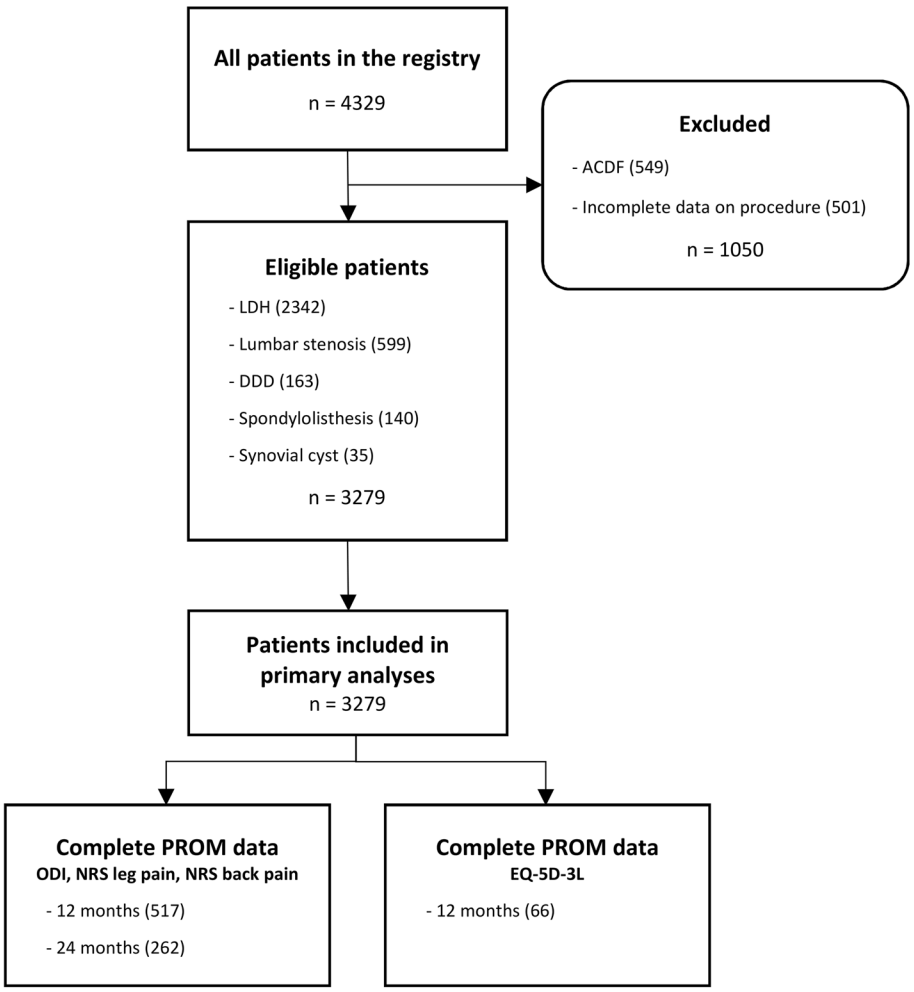


**Fig. 1** Flowchart demonstrating the flow of patients throughout this analysis

**Table 1** Baseline patient characteristics stratified by gender

| Parameter | Gender | |
|---|---|---|
| | Male | Female |
| Age, mean ± SD (years) | 47.7 ± 13.3 | 48.5 ± 13.5 |
| Active smoker, *n* (%) | 460 (34%) | 304 (26%) |
| BMI, mean ± SD (kg/m$^2$) | 25.8 ± 3.0 | 25.1 ± 3.7 |
| Indication, *n* (%) | | |
| LDH | 1244 (72%) | 1098 (71%) |
| Lumbar stenosis | 327 (19%) | 272 (18%) |
| DDD | 81 (4.7%) | 82 (5.3%) |
| Spondylolisthesis | 65 (3.7%) | 75 (4.9%) |
| Synovial cyst | 19 (1.1%) | 16 (1.0%) |
| ASA Class, *n* (%) | | |
| Class I | 968 (64%) | 794 (59%) |
| Class II | 531 (35%) | 551 (41%) |
| Class III | 7 (0.5%) | 4 (0.3%) |

*BMI* Body mass index; *LDH* lumbar disc herniation; *DDD* degenerative disc disease; *ASA* American society of anesthesiologists

A detailed overview of adverse events and reoperations is provided in Table 3. There were no relevant differences among genders in terms of both complication (5.6 vs. 4.5%, $p = 0.15$) or reoperation rates (11 vs. 8.7%, $p = 0.08$). This applied for reoperations at the index level (5.6 vs. 4.9%, $p = 0.40$) and at other levels (4.5 vs. 3.5%, $p = 0.11$).

## Absolute outcome measures

In terms of ODI, female patients presented with significantly worse baseline functional disability (Δ 4.6, 95% CI 2.6–6.6, $p < 0.001$, Fig. 2), and reported higher degrees of functional disability at 6 weeks, 12 and 24 months after surgery (Table 4). Specifically, 12-month ODI scores, the primary endpoint, were significantly higher (Δ 5.5, 95% CI 3.3–7.6, $p < 0.001$). Female patients also experienced more baseline leg (Δ 0.3, 95% CI 0.1–0.6, $p = 0.016$) and back pain (Δ 0.4, 95% CI 0.1–0.8, $p = 0.006$) NRS scores. Except for early 6-week leg pain, female patients showed considerably higher pain scores during the follow-up period (Fig. 3). Baseline HRQOL measures were

**Table 2** Operative parameters stratified by gender

| Parameter | Gender | | *p* |
|---|---|---|---|
| | Male | Female | |
| Index level, *n* (%) | | | – |
| L1–L2 | 2 (0.1%) | 3 (0.2%) | |
| L2–L3 | 57 (3.3%) | 27 (1.8%) | |
| L3–L4 | 196 (11%) | 155 (10%) | |
| L4–L5 | 735 (43%) | 631 (41%) | |
| L5–S1 | 732 (43%) | 717 (47%) | |
| Right-sided, *n* (%) | 582 (43%) | 505 (44%) | – |
| Midline, *n* (%) | 47 (3.5%) | 56 (4.8%) | – |
| Bilateral, *n* (%) | 32 (2.4%) | 37 (3.2%) | – |
| Procedure, *n* (%) | | | – |
| tMD | 1316 (76%) | 1104 (72%) | |
| Decompression | 275 (16%) | 246 (16%) | |
| MI-TLIF | 62 (3.6%) | 68 (4.4%) | |
| MI-PLIF | 41 (2.4%) | 68 (4.4%) | |
| ALIF | 30 (1.7%) | 33 (2.1%) | |
| AxiaLIF | 12 (0.7%) | 24 (1.6%) | |
| Intraoperative parameters, mean ± SD | | | |
| Surgical time (min.) | 37.2 ± 38.6 | 40.5 ± 42.1 | 0.02* |
| Length of stay (d) | 1.1 ± 0.6 | 1.2 ± 0.6 | < 0.001* |
| Estimated blood loss (mL) | 292.5 ± 247.8 | 258.9 ± 236.6 | < 0.001* |

*tMD* Tubular microdiscectomy; *MI-TLIF* minimally invasive transforaminal lumbar interbody fusion; *MI-PLIF* minimally invasive posterior lumbar interbody fusion; *ALIF* anterior lumbar interbody fusion; *Axi-aLIF* transaxial lumbar interbody body fusion

*$p \leq 0.05$

**Table 3** Complications and reoperations stratified by gender

| Parameter | Gender | | *p* |
|---|---|---|---|
| | Male | Female | |
| Complications, *n* (%) | 97 (5.6%) | 69 (4.5%) | 0.15 |
| Incidental durotomy | 80 (4.6%) | 51 (3.3%) | |
| Paresis | 7 (0.4%) | 2 (0.1%) | |
| Wound infection | 2 (0.1%) | 4 (0.3%) | |
| Spondylodiscitis | 2 (0.1%) | 4 (0.3%) | |
| Haematoma | 1 (0.1%) | 2 (0.1%) | |
| Bleeding | 3 (0.2%) | 1 (0.1%) | |
| Total reoperations, *n* (%) | 182 (11%) | 134 (8.7%) | 0.08 |
| Reoperations at index level, *n* (%) | 97 (5.6%) | 76 (4.9%) | 0.40 |
| Stenosis | 13 (0.7%) | 3 (0.2%) | |
| LDH | 72 (4.1%) | 54 (3.5%) | |
| Spondylolisthesis | 11 (0.6%) | 17 (1.1%) | |
| Implant failure | 1 (0.1%) | 2 (0.1%) | |
| Reoperations at another level, *n* (%) | 78 (4.5%) | 54 (3.5%) | 0.11 |
| Stenosis | 12 (0.7%) | 9 (0.6%) | |
| LDH | 50 (2.9%) | 37 (2.4%) | |
| Spondylolisthesis | 16 (0.9%) | 8 (0.5%) | |

*LDH* Lumbar disc herniation

*$p \leq 0.05$

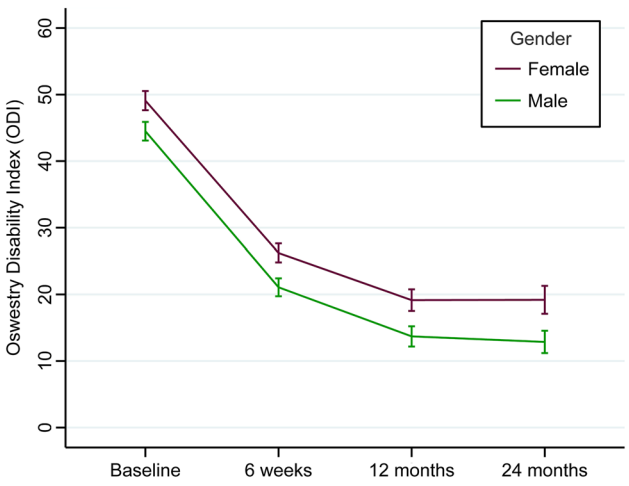


**Fig. 2** Oswestry disability index (ODI) scores during the follow-up period. Error bars represent 95% CI. *ODI* Oswestry disability index; *CI* confidence interval

comparable among genders (both $p > 0.05$). At both 6 weeks ($p = 0.027$) and 12 months ($p = 0.016$), female patients presented with lower HRQOL on the EQ-5D index

**Table 4** Absolute patient-reported outcome measures stratified by gender

| Absolute score | Gender | | *p* |
|---|---|---|---|
| | Male | Female | |
| ODI | | | |
| Baseline | 44.5 ± 18.2 | 49.1 ± 17.6 | < 0.001* |
| 6 weeks | 21.1 ± 17.0 | 26.2 ± 17.2 | < 0.001* |
| 12 months | 13.7 ± 16.9 | 19.2 ± 17.8 | < 0.001* |
| 24 months | 12.9 ± 14.8 | 19.2 ± 17.7 | < 0.001* |
| NRS leg pain | | | |
| Baseline | 7.0 ± 2.3 | 7.3 ± 2.3 | 0.016* |
| 6 weeks | 2.1 ± 2.5 | 2.1 ± 2.5 | 0.84 |
| 12 months | 1.9 ± 2.6 | 2.6 ± 2.9 | < 0.001* |
| 24 months | 1.9 ± 2.7 | 2.6 ± 3.0 | 0.001* |
| NRS back pain | | | |
| Baseline | 5.3 ± 2.9 | 5.8 ± 2.8 | 0.006* |
| 6 weeks | 2.9 ± 2.4 | 3.4 ± 2.5 | < 0.001* |
| 12 months | 2.8 ± 2.6 | 3.3 ± 2.8 | 0.005* |
| 24 months | 2.5 ± 2.6 | 3.1 ± 2.9 | 0.010* |
| EQ-5D index | | | |
| Baseline | 0.42 ± 0.30 | 0.39 ± 0.32 | 0.288 |
| 6 weeks | 0.75 ± 0.22 | 0.69 ± 0.24 | 0.027* |
| 12 months | 0.84 ± 0.20 | 0.78 ± 0.24 | 0.016* |
| EQ-VAS | | | |
| Baseline | 51.7 ± 18.2 | 48.6 ± 17.3 | 0.08 |
| 6 weeks | 69.4 ± 16.6 | 68.5 ± 15.6 | 0.61 |
| 12 months | 75.3 ± 17.4 | 71.8 ± 16.7 | 0.07 |

Values are provided as mean ± standard deviation

*ODI* Oswestry disability index; *NRS* numeric rating scale; *EQ-VAS* EuroQol visual analogue scale

*$p \leq 0.05$

(Fig. 4). There were no differences among EQ-VAS scores at 6 weeks ($p = 0.61$) and 12 months ($p = 0.07$).

### Relative outcome measures

Change scores for ODI at 12 months were equal among male and female patients (Δ −1.6, 95% CI −5.5–2.3, $p = 0.43$). Overall, there were no significant differences between genders in the degree of improvement from baseline in any of ODI, NRS leg and back pain, EQ-5D index, and EQ-VAS, at any of the follow-up dates (all $p > 0.05$, Table 5). Clinical success, as defined by a 12-month improvement equal to or greater than the MCID of 30% from baseline in ODI scores, was achieved in 82% of men and 79% of women ($p = 0.34$).

## Discussion

In an analysis of 3279 patients from a prospective registry, we compared female and male patients to identify any relevant differences in outcomes and adverse events. Complication and reoperation rates were equal among both genders, but female patients experienced higher estimated blood loss as well as prolonged surgical times and length of stay perioperatively. In terms of patient-reported outcomes, female patients generally presented with a worse preoperative status than men, but subsequently demonstrated the same degree of improvement even at long-term follow-up.

Considering gender-related differences in the prognosis and treatment of patients with spinal disorders is attracting major interest [1, 16]. Knowledge about the effect size and mechanisms of such differences is valuable and may lead to enhanced shared decision-making and greater patient satisfaction [1].

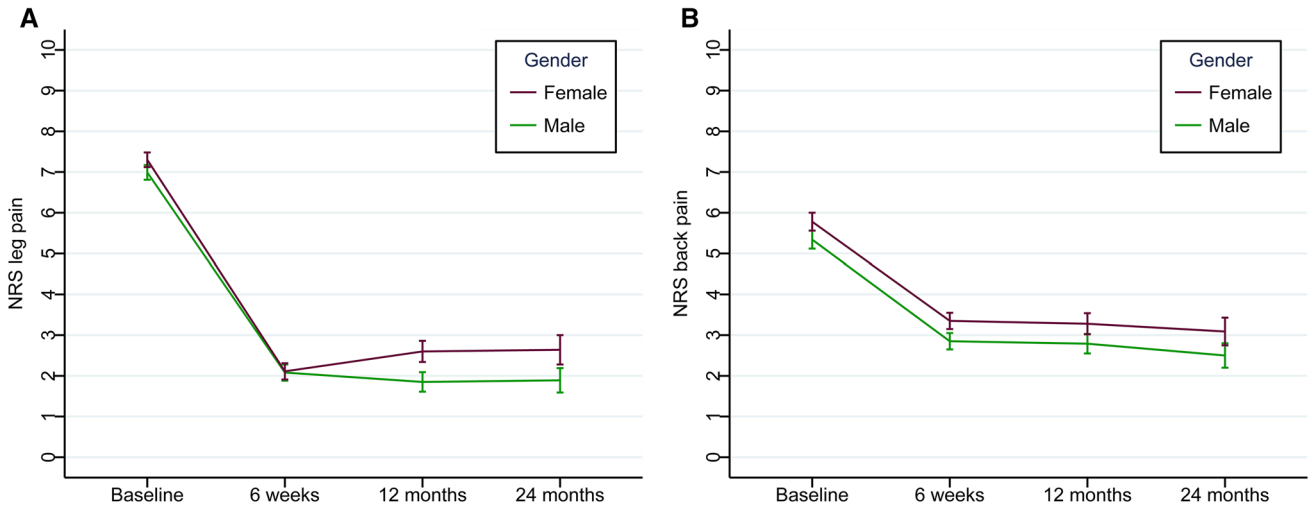


**Fig. 3** Graphic representation of the NRS leg pain and NRS back pain. Error bars represent 95% CI. *NRS* Numeric rating scale; *CI* confidence interval

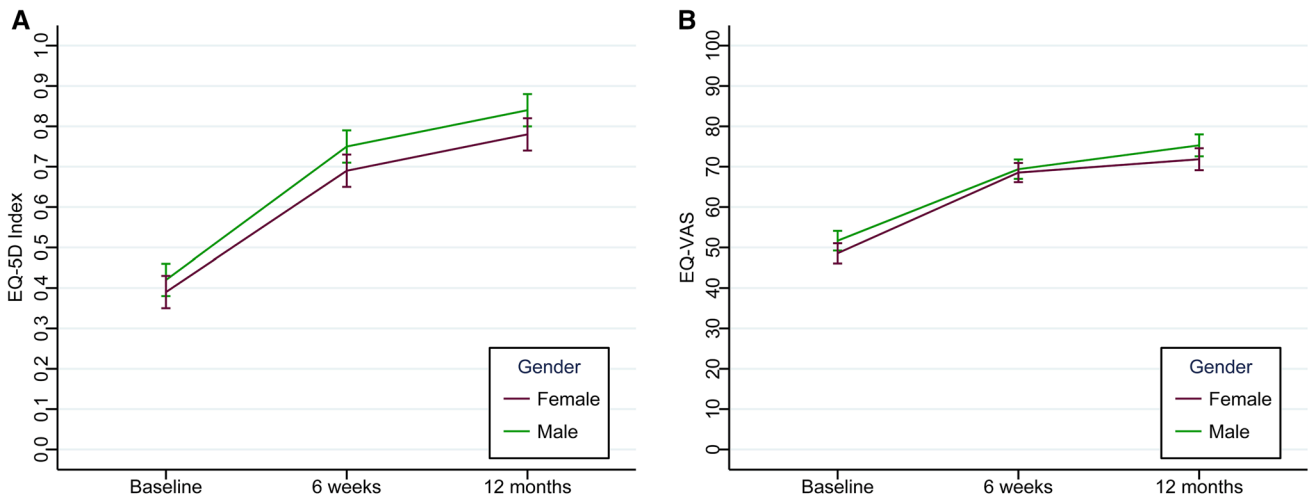


**Fig. 4** Graphic representation of the EQ-5D-3L measures during follow-up period. Error bars represent 95% CI. *VAS* Visual analogue scale; *CI* confidence interval

**Table 5** Degree of improvement from baseline (change score) in patient-reported outcome measures stratified by gender

| Change score | Gender | | *p* |
|---|---|---|---|
| | Male | Female | |
| ODI | | | |
| 6 weeks | −23.9 ± 22.1 | −21.7 ± 22.3 | 0.15 |
| 12 months | −31.2 ± 23.4 | −29.6 ± 21.9 | 0.43 |
| 24 months | −30.2 ± 23.3 | −31.1 ± 22.6 | 0.75 |
| NRS leg pain | | | |
| 6 weeks | −5.1 ± 3.2 | −5.4 ± 3.2 | 0.22 |
| 12 months | −5.1 ± 3.4 | −4.6 ± 3.4 | 0.13 |
| 24 months | −4.9 ± 3.4 | −4.5 ± 3.7 | 0.42 |
| NRS back pain | | | |
| 6 weeks | −2.4 ± 3.3 | −2.5 ± 3.3 | 0.70 |
| 12 months | −2.5 ± 3.5 | −2.3 ± 3.4 | 0.61 |
| 24 months | −2.7 ± 3.3 | −2.4 ± 3.4 | 0.43 |
| EQ-5D index | | | |
| 6 weeks | 0.34 ± 0.36 | 0.30 ± 0.36 | 0.36 |
| 12 months | 0.38 ± 0.31 | 0.35 ± 0.37 | 0.76 |
| EQ-VAS | | | |
| 6 weeks | 17.7 ± 21.3 | 18.3 ± 21.3 | 0.83 |
| 12 months | 27.0 ± 20.1 | 16.0 ± 28.0 | 0.07 |

Values are provided as mean ± standard deviation

*ODI* Oswestry disability index; *NRS* numeric rating scale; *EQ-VAS*, EuroQol visual analogue scale

*$p \leq 0.05$

The precedent that female patients do somewhat worse after spine surgery has been set by earlier studies [19]. Ekman et al. [8] indicated that female patients with isthmic spondylolisthesis presented with worse preoperative status and that female sex and non-working status were the best predictors of unfavourable outcome. Similarly, Gehrchen et al. [9] found that women undergoing fusion surgery report lower satisfaction, slower return to work, and increased consumption of analgetics. These findings directly contradict our results, as well as those indicated by the more recent literature.

We found that female patients presented with worse preoperative pain and functional disability, but similar HRQOL compared to men. Postoperatively, these absolute differences among genders persisted for pain and disability. In addition, women reported a lower HRQOL as measured by the EQ-5D index at follow-up. However, when looking at the degree of improvement from baseline, there were no differences between genders, indicating that both male and female patient profit to the same degree from surgery for degenerative lumbar spinal pathologies. These findings are corroborated by recent studies.

A large analysis by Pochon et al. [16] looking at the Core Outcome Measures Index (COMI) found that, while reporting worse values preoperatively, female patients undergoing surgery for lumbar disc herniation (LDH), stenosis, or spondylolisthesis profit to the same degree as their male counterparts. Moreover, they found that gender had no effect on the likelihood of achieving a favourable outcome in a multivariate analysis. Strömqvist et al. [12, 13, 18] have performed multiple registry-based analysis on the influence of gender on LDH. They determined that women consume more analgetics, report more pain and disability, as well as a lower HRQOL, both preoperatively and at follow-up [12, 18]. These effects were somewhat less pronounced in the paediatric population from the same registry [13]. Triebel et al. [19] analysed patients undergoing lumbar fusion, and established that, although starting off with worse symptoms, female patients showed higher change scores and were

even more likely to achieve MCID. Interestingly, the effect size of gender was smaller on return to work and HRQOL, which correlates to the findings of our analysis. For patients with degenerative disc disease undergoing lumbar fusion, van Hooff et al. [21] identified female gender as a reliable predictor of achieving MCID. Chan et al. [14] even found that female sex was the best predictor of satisfaction after surgery for low-grade spondylolisthesis. Gulati et al. [15] determined that female patients showed higher ODI change scores at 1 year postoperatively in adolescents with sciatica. Gautschi et al. [25] also demonstrated a similar degree of improvement among genders, even though female patients reported worse absolute symptom severity preoperatively and postoperatively.

Multiple explanations for gender-related differences in pain reporting have been considered throughout the years [5, 6]. Because women report worse symptoms preoperatively, it is conceivable that an objectively more advanced stage of disease in female patients may contribute to gender-related outcome differences, as Katz et al. have demonstrated [10, 12]. The physiological differences among genders are still poorly understood [1, 5, 6]. Divergences in disease susceptibility, inflammation, and analgetic drug effectiveness may play a role [1, 4–7, 26]. While there is no definite proof that these differences are mediated by hormonal factors [6], psychological differences may be of significance. Robinson et al. [26] demonstrated that men are less inclined than women to report pain on questionnaires. Psychometric properties of questionnaires and the resulting reporting bias may thus explain part of the difference in pain scores among genders.

The perception of pain can be estimated by quantitative sensory testing (QST). Myers et al. [27] tested the hypothesis that gender-related differences in pain experience may be related to differences in blood pressure change. While they refuted this hypothesis, they were able to demonstrate a markedly lower pain threshold in women. Tschugg et al. [2, 3] used QST to demonstrate that this discrepancy in pain perception is not only present in healthy individuals, but also in LDH patients. They concluded that heat and pressure pain thresholds were lower in female patients preoperatively and that these differences disappeared for heat perception postoperatively. In a study on pain sensitivity in degenerative disc disease, Kim et al. [7] found that women presented with higher VAS and ODI scores, as well as worse HRQOL and a higher pain sensitivity. After adjustment for pain sensitivity, these differences in symptom severity ceased, further underlining the importance of pain thresholds in outcome measurement.

The timing, type, and exact method of outcome measurement is another major contributor to the effect size of gender on PROMs [10, 23, 25]. Generally, we found much smaller intergroup differences for HRQOL than for functional disability and pain severity. In terms of timing, recalled pain severity vastly underestimates inter-gender differences as compared to actual measurement [10]. Outcomes can be assessed in a multitude of dimensions. In the lumbar spine, outcome dimensions that are commonly assessed are pain severity, functional disability, and HRQOL. Recently, objective tests that measure objective functional impairment (OFI) have gained interest, partly because they promise to be more robust against confounders such as mental status or gender. Gautschi et al. [25] demonstrated that women reported worse preoperative disease states as assessed by conventional questionnaires on pain and disability, but that the degree of OFI was equal among genders. Staartjes and Schröder [28] also determined that gender did not influence OFI. Thus, objective functional testing may constitute a valid alternative for clinical assessment that is independent of gender influence.

In terms of complications and reoperations, we found no differences between genders. This is corroborated by a comprehensive meta-analysis of 45 studies by Schoenfeld et al. [11], which concluded that the complication rate was equal among genders, although men were more prone to postoperative mortality than women.

From our data, as well as the recent literature, it seems clear that women report more absolute pain and functional disability preoperatively and at follow-up, but that the degree of improvement during the follow-up period is equal among genders. In the peer-reviewed literature, improvement and absolute disease severity are sometimes used interchangeably, which might lead to confusion when gender is being employed as a risk factor for outcome. Therefore, it is important that it is always clearly stated if absolute measurements or change scores are being discussed. Our results indicate that female patients can expect to profit from surgery for degenerative lumbar spinal diseases to the same degree as male patients. A systematic review and meta-analysis of the literature on this topic may be warranted.

## Limitations

Our study is primarily limited by its retrospective nature. Although all data were collected in a prospective registry, events were captured systematically, and all patients with sufficient data were included, selection bias and underreporting of adverse events cannot be ruled out. Furthermore, all data stems from a single centre and only two senior surgeons, possibly creating centre bias. Not all patients completed sufficient PROM questionnaires, which may result in reporting bias. However, the gender distribution of patients included in the PROM analysis was the same as in the original registry, indicating that dropout was equally divided among genders. Because only relatively healthy patients were included, as demonstrated by the relatively

low American Society of Anesthesiologists grades, our findings may not be extrapolated to patients with severe systemic comorbidities, or to very elderly patients. Lastly, we were unable to assess gender differences in return to work and objective functional impairment.

## Conclusions

In an analysis of a large prospective registry, both genders showed a comparable degree of improvement from baseline and a similar rate of clinical success. However, female patients present with worse pain and function preoperatively and continue to report more severe symptoms during the follow-up period. Female patients may thus fare worse in terms of absolute scores, but enjoy the same benefit from surgery in relative terms. These data contribute to the limited evidence in the literature on the role of gender in the perception and treatment of back pain and sciatica. This may prove valuable in clinical shared decision-making.

### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (Medical Research Ethics Committees United, Registration Number: W16.065) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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