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Research paper

The SpinalMeter biometrical assessment to improve posture diagnosis in school-age girls: a validation study

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Abstract

Introduction: Assessing spinal deformities using an X-ray radiation is the method of choice for posture diagnosis. It is also used for the evaluation of the degree of correction, brace fit, and spinal balance as well as for further management decisions. However, multiple X-ray exposures during control visits could be too burdensome for children.

Aim: The aim of this study was to investigate the precision and repeatability of measurement of the variables obtained by a fast, simple postural evaluation in children by the SpinalMeter.

Material and methods: The measurements of the angle of trunk rotation (ATR) and SpinalMeter posture assessments were performed 8 to 10 times in a short period of time (6 s). The overall of 300 photos (SpinalMeter) and 1020 measurements (asymmetry, distance between anthropometric points as well as ATR) were obtained from 6 girls (8–15 years old). The validation study comprised of the repeatability, interclass correlation coefficient (Qw) and relative standard deviation (rSD) measurements.

Results and discussion: The measurements of the distance between acromion–popliteal fossa, acromion–iliac crest, and acromion–posterior superior iliac spine obtained by SpinalMeter were clearly repeatable (Qw > 0.9). The scapular and pelvic asymmetry in standing and sitting positions were highly repeatable and had low rSD (e.g. for scapular asymmetry 5.6%–80.3%; Qw > 0.8).

Conclusions: The precise and reliable postural biometrical measurements were performed by SpinalMeter in the case of the distance between anthropometric points and asymmetry of pelvis and scapula. These measurements could be useful in the assessment of girl's posture when visiting the pediatrician.

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1. INTRODUCTION

1.1. Available knowledge

The implementation of new diagnostic tool in order to improve the quality of a newly designed method and safety of patients' needs specific validation process. A gap between practice at the local level and the achievable evidence-based standard is still a rising problem. There is quite small number of recent scientific research checking the specific posture assessment methods.[1] As a result, these methods after validation could act as a faster and less invasive alternative for the routinely used methods. Moreover, the sitting position has not been widely used during posture diagnosis yet. It was shown that the sitting position with bad movement pattern is associated with asymmetries of the trunk and scoliosis, whole body imbalance and can cause a decrease in lumbar lordosis and kyphosis of a child's entire spine.[2,3] This study opens the new field in the healthcare improvement because of not only standing but also the sitting position used in the validation and assessment of posture in school-age girls.

1.2. The method validation process

Validation of the research method is the process of providing objective proof, which is designed to confirm the suitability of the method for a given application.[4] The validation tests and checking the method is needed to obtain the capabilities (consistent with what the application requires) and limitations of the research method. Furthermore, the validation process is an important source of input data which estimates the measurement uncertainty and is the starting point for the construction of the quality control program.[5] Results from method validation can be used to judge the quality, reliability and consistency of the results. Validation is an integral part of any good analytical practice.[6]

All research methods need to be validated or revalidated: before their introduction into routine use; whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix); as well as whenever the method is changed and the change is outside the original scope of the method.[7,8]

The validation process for analytical methods is well known and the specific variables have been described.[9] The validation process for pharmaceutical applications is well described in the International Conference on Harmonization.[8] It is based on the evaluation of the selectivity/specificity, precision, reproducibility, accuracy and recovery of the research method etc.[10] However, in the rehabilitation and public health studies only selected validation variables could be applicable. Thus, not all of them have been described below.

1.3. Precision of the method

The two most common precision measures are 'repeatability' and reproducibility.' Repeatability (the smallest expected precision) is determined by calculating the variability of the results of method carried out under the same conditions (by a single analyst on a one piece of equipment over a short timescale).[11] Precision is usually stated in terms of standard deviation (SD) or relative standard deviation (rSD).[11]

1.4. Variability of the results

The measurements of the variability of the results determine the differences between individual test results and the value of the arithmetic mean. This is mostly often described by:

- variance (V),
- standard deviation (SD),
- relative standard deviation (rSD), and
- range.[11,12]

1.5. SpinalMeter method for the determination of postural status of children

Evaluating postural status of children (e.g. with scoliosis) is mainly based on radiographic examination.[13] On the other hand, an X-ray examination in children should be performed only for a valid medical reason and with minimal radiation dose, which is necessary to achieve a diagnostic high quality result.[14] Moreover, it should be performed in accordance with the American College of Radiology and the Society for Pediatric Radiology Practice Parameter for General Radiography.[15] Thus, it is advised to reduce the radiation hazard by performing an X-ray projection only in the area of interest, and invasive follow-up should be discarded when possible.[16]

It is well known that X-ray imaging of scoliosis is the most reliable and the most commonly used method for the initial diagnosis. However, the multiple X-ray exposures during control visits would be too burdensome for the children.[17,18] On the other hand, the newest systems using e.g. convolutional neural networks were developed and proposed for clinical assessment. However, these studies need more clarification.[19]

It should be noted that previous recommendations showed that it should be a routine to screen children for scoliosis, because there is a large number of school children suffering from adolescent idiopathic scoliosis (AIS). However, the specific methods should be used because of the controversy shown in the context of school screening programs.[20]

In this study, a fast and simple postural biometrical assessment using SpinalMeter in standing and sitting positions has been proposed for the posture evaluation in children with scoliosis. The need for this study was dictated by the recommendations to perform high quality research on innovative, non-invasive scoliosis treatment as well as fast, reproducible diagnostic methods.[21]

2. AIM

The aim of this study was to investigate: (a) validation of spinal posture results assessed by SpinalMeter; (b) the precision and repeatability of the SpinalMeter diagnostic tool; (c) the variability of the results obtained by SpinalMeter.

3. MATERIAL AND METHODS

3.1. Study population

The measurements of the angle of trunk rotation (ATR) and SpinalMeter posture assessment were performed 8 to 10 times in a short period of time (6 s) by 2 professional experts. The overall of 300 photos for SpinalMeter assessment were made. The SpinalMeter method validation was based on the assessment of posture in 6 school-age girls with scoliosis aged between 8 and 15 years old (12.2 ± 3.3 years old) diagnosed and treated at the Humanus Centre of Rehabilitation in Olsztyn and in Regional Specialized Children's Hospital in Olsztyn, Poland (Table 1).

The choice of girl participants in this study was dictated by the general knowledge about the prevalence of scoliosis in females vs. males. It is known that girls grow very rapidly until their first menstrual period. AIS commonly affects girls with a ratio of 1.5 : 1 for females and 3 : 1 for males. This ratio increases substantially with increasing age.[22] According to the American Academy of Orthopaedic Surgeons, idiopathic scoliosis occurs 10 times more often in girls than it does in boys over the age of 10.[23] Moreover, some hormones could also be associated with an increased risk for scoliosis in girls. Some research showed that girls who have higher amounts of leptin hormone and sympathetic nervous system activity are more likely to have scoliosis.[24] Moreover, the abnormal leptin bioavailability with increased levels of the soluble leptin receptor (sOB-R) were reported in girls with AIS.[25] Furthermore, higher ghrelin levels (6.33 ± 2.46 vs. 4.46 ± 2.02 ng/mL, $P < 0.05$) were found in all AIS patients, which was also associated with higher curve progression rates.[26] Not only are girls more likely to develop scoliosis, but they are also more likely to develop severe curves as well.[22]

The inclusion criteria consisted of: scoliosis confirmed in the clinical and radiological assessment, Cobb's angle between 10° and 15°, Risser sign less than 4. Participants were either premenarcheal or less than 3 years post-menarcheal, had no history of brace treatment, no co-morbidities affecting the course of scoliotic deformation such as genetic defects, neuromuscular disorders, metabolic disorders, history of severe trauma.

The qualification of girl's posture as scoliosis were based on the guidelines of Society on Scoliosis Orthopaedic and Rehabilitation Treatment. If the Cobb angle was at least 10° the scoliosis was diagnosed.[27]

3.2. SpinalMeter equipment and requirements

The SpinalMeter system consisted of:

- a personal computer system (Acer Aspire E17; CPU IntelCore i5-5200U 2.2 GHz, 64 bit and 8 GB RAM;
- operating system Windows 10 Education;
- monitor with 1024 × 768 pixel resolution);
- a stand with the camera (Canon EOS 1200D with 18 megapixel CMOS), a 'calibration' platform (42 × 42 cm), and
- a stand-platform connector (the distance between the girl's heel and the camera was 243 cm).

SpinalMeter equipment was provided by the IT Medical Group.

3.3. Interventions

This study consisted of both radiographic and anthropometric measurement (initial diagnosis). Basic posture diagnosis consisted of: anthropometric measurements, an posterior-anterior X-ray imaging, SpinalMeter posture assessment and the ATR assessment.

3.3.1. Anthropometrics

Children's body weight (after removal of shoes and heavy clothing) was measured to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm using a patient weighing scale with a height rod (Seca 217, Seca, Polska). Body mass index (BMI) was calculated as weight in kilograms, divided by height in meters squared, rounded to the nearest tenth. Spine length was measured using standard measuring tape.

3.3.2. An X-ray imaging

An posterior–anterior X-ray (PA) image of spine, the iliac crests and femoral heads in standing position was performed. PA Cobb's angles were measured using Cobb's method from standing PA radiographs.[28]

The Risser stage was assessed according to the European Risser Staging System.[29] An X-ray of the girl's spine was taken a day before the SpinalMeter examination.

3.3.3. SpinalMeter calibration and posture assessment

The SpinalMeter calibration was performed prior to taking girl's measurement but always on the same day.

Anthropometric points on the body defined by landmarks (Maestrale Italy markers, Terni, Italy) that point

Table 1. The initial characteristics of the population studied.

Patient code	Height, m	Body mass, kg	BMI, kg/m^2	Spine length, cm	Cobb angle, °	ATR, °	Location	Menstruation
BH	1.3	27.0	15.6	42.5	10.0	3.0	L1	No
MJ	1.4	34.0	16.3	47.0	15.0	3.0	Th7	No
FK	1.7	52.0	17.2	54.0	10.0	3.5	Th7	Yes
SM	1.6	50.0	19.1	51.0	10.0	4.0	L1	Yes
RM	1.7	56.0	20.3	50.0	11.0	2.0	L1	Yes
SP	1.7	75.0	27.1	50.0	13.0	3.5	L1	Yes
Mean	1.6	49.0	19.3	49.1	11.5	3.2	–	–
SD	0.16	16.99	4.23	3.93	2.07	0.68	–	–

Comments: L – lumbar spine; Th – thoracic spine.

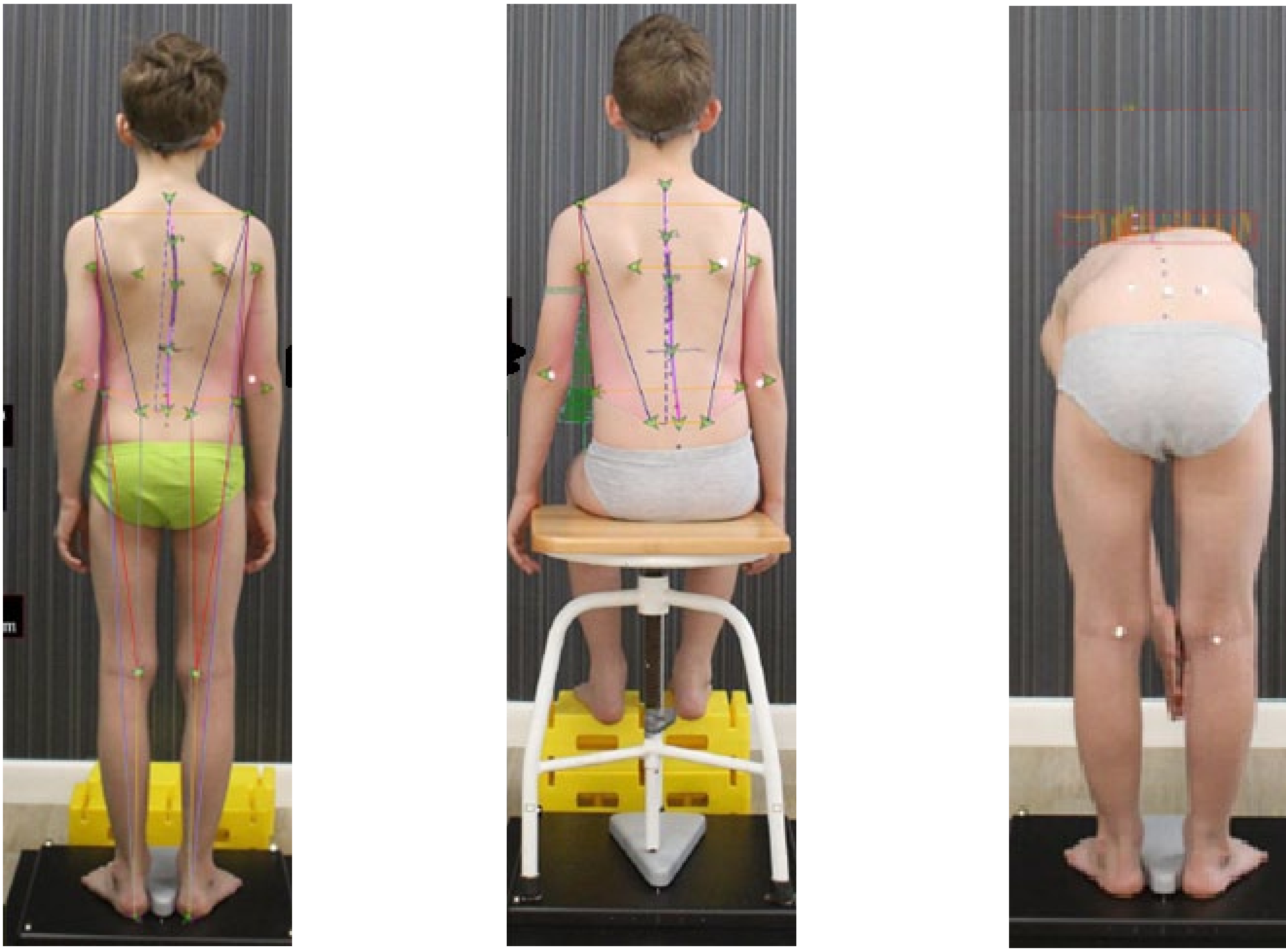

Figure 1. Patient (code: SZ, 10 years old) with the anatomic indexing points during standing, sitting position and forward bending test (Gibbo).

out the spine's position and limb's length and others readings e.g. surface of the triangles of the size were used for a complete evaluation of the subjects. The precise fit of the landmarks was done by specific HSL Linear Correction. The anatomic indexing points have been shown in Figure 1. The specific anatomic points were described previously.[30] Additionally, in the position of landmarks the point on the body was drawn with marker pen. In this study, only selected positions of the body as well as asymmetry and length measurements were taken into account.

After putting the reflective markers on the girls' body, the digital photo examination was performed, in the frontal plane with the arms hanging at the side of the body and the feet and knees together, according to the natural stance of the patient. The children feet location was ensured by the specific 'calibration' platform. The processing of the image has been performed in SpinalMeter Evolution v6.14.

In this study, the standing as well as the sitting and forward bending (Gibbo) positions were taken into account. The following 8 to 10 measurements in a short period of time (6 s) were taken by 2 professional experts.

3.3.4. The measurement of the ATR

The assessment of scoliosis included the Adams forward bending test, and the objective measurement of the ATR using scoliometer made by 2 trained evaluators/diagnosticians.[31] It is known that the measured rib hump is directly related to spinal rotation and rib deviations.[32]

The reliability of the measurements obtained with the scoliometer was determined as very good to excellent in a previous study.[33] Thus, in the studied group the scoliometer was used to analyse the axial rotation of the trunk (i.e. ATR). The scoliometer was placed over spinous processes of the back and was drawn along them to measure the axial trunk rotation.

3.3.5. The statistical study of the interventions

Statistical analysis was performed using Statistica 13.0 software (TIBCO Software Inc. 2017, Krakow, Poland)[34] and IBM SPSS 25 software (SPSS Polska, 2013–2017).[35]

The validation of the measurement error was based on the estimation of the repeatability coefficient of a feature of same person (number of patients $n = 6$) in subsequent repetitions (8–10, based on photographs taken in a row). The repeatability coefficients were determined using the intra-class correlation coefficient (Q_w) expressed by the quotient of variance components:

$$Q_w = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2},$$

where σ_a^2, was placed by the the assessment of component

$$\sigma_a^2 = \frac{S_a^2 - S_e^2}{k},$$

and a σ_e^2 was placed by the assessment of component S_e^2.[36–38]

The intra-class correlation coefficient measures the degree of correlation of elements of the same class. Thus, it could be used to assess the repeatability of measurements.[37,38] The mean squares of inter- and intra-group variability were estimated in a mixed model of variance analysis with a highlighted random factor (number of photo). In this study, the rSD was calculated for a series of measurements taken on subsequent photos.

In addition, it was assumed that none of the statistically significant differences should be shown between the estimators of measurement diversity for the examined group of people in subsequent assessments (based on photos taken in a row). For subsequent repetitions of measurements, the mean value and its standard errors (SE) were determined, and the significance of differences in the repeated measures ANOVA was tested. The sphericity assumption in model of variance could not be confirmed due to the experimental design. Thus, the corrected values of type I error rate were calculated as the correction of the lower-bound estimate epsilon (ε) using the Greenhouse–Geiser and Huynh-Feldt method, and then correcting the probability value according to the suggestion of Howell (2002) and Field (2013).[35,39]

4. RESULTS

4.1. Asymmetry measurements and the correlation between standing and sitting positions

The comparison of asymmetry measurements between standing and siting positions have been shown in Table 2. The highest asymmetry was observed in scapula position in sitting as well as in standing positions. High values of posterior superior iliac spine (PSIS) asymmetry were observed only in 1 girl in sitting position. The lowest values have been indicated in pelvic asymmetry in standing position and in shoulder asymmetry in sitting position.

4.2. The validation of the results obtained from SpinalMeter postural biometrical assessment

The validation process revealed that the length measurements (Table 3) obtained by SpinalMeter were more reliable than anthropometric points asymmetry measurements (Table 4). In this study, the high repeatability of measurements of variables was obtained for acromion–popliteal fossa (ac-PF), acromion–iliac crest (ac-IC) and acromion–PSIS (ac-PSIS) distance for both right and left side of the body (Q_w for these measurements was above 0.9). The variability of these measurements taken on subsequent photos was within the statistical error range – rSD below 1% (Table 4). It was shown that this method is suitable for the measurement of different anthropometric points of the body.

Lower repeatability of measurement were shown for variables related to the assessment of the degree of asymmetry (shoulder, PSIS), depending on the position in which the photos were taken (Table 4). The lowest repeatability was shown for the distance of the transverse process from midline in the Gibbo position (Q_w = 0.715). The total variability of measurements was high, and for some people reached 174.12%. Very high variability was also assessed for measurements of the shoulder asymmetry in the sitting position and the PSIS in the standing position (Table 3) Better results of repeatability of measurements were shown for the scapular and pelvic asymmetry, regardless of the position in which the photos were taken (Table 3). In all cases, no differences were found.

5. DISCUSSION

5.1. Summary

In this study, the non-invasive method for the assessment of body posture has been proposed. It has been shown pre-

Table 2. The asymmetry measurements (°) obtained by SpinalMeter postural diagnosis.

Variable	Value	Standing		Sitting	
		Mean	SD	Mean	SD
Shoulder asymmetry	Minimum	0.27	0.30	0.22	0.19
	Maximum	2.67	0.95	1.81	0.57
Scapular asymmetry	Minimum	1.25	1.20	1.64	1.31
	Maximum	7.50	0.59	6.10	0.34
Pelvic asymmetry	Minimum	0.13	0.12	0.91	0.25
	Maximum	3.41	0.24	3.61	0.65
PSIS asymmetry	Minimum	0.40	0.26	0.61	0.37
	Maximum	1.46	0.51	5.92	0.76

Comments: The highest and the lowest values has been indicated by bold.

Table 3. The repeatability coefficients of selected variables, the range of diversity of the coefficient variation of measurement, range of estimation of average measurements for the examined group of people in subsequent repetitions and analysis of the variation of average estimation.

Variable	Q_w	rSD	Range of means estimation[1]	P^2	P^3
Standing					
Shoulder asymmetry	0.807	24.7%–112.8%	0.83 (0.300) – 1.45 (0.604)	0.475	0.613
Scapular asymmetry	0.840	7.8%–96.1%	3.64 (0.853) – 4.99 (0.766)	0.330	0.294
Pelvic asymmetry	0.936	7.1%–91.4%	1.20 (0.574) – 1.62 (0.431)	0.352	0.339
PSIS asymmetry	0.461	34.8%–65.6%	0.57 (0.255) – 1.27 (0.365)	0.393	0.418
Sitting					
Shoulder asymmetry	0.453	27.9%–87.6%	0.55 (0.241) – 1.39 (0.431)	0.275	0.205
Scapular asymmetry	0.880	5.6%–80.3%	3.15 (0.710) – 3.98 (0.556)	0.716	0.925
Pelvic asymmetry	0.965	11.0%–50.9%	1.63 (0.370) – 1.97 (0.618)	0.777	0.968
PSIS asymmetry	0.896	12.7%–72.6%	1.94 (0.750) – 2.84 (0.932)	0.391	0.403
Gibbo					
The distance of spinous process from midline	0.715	47.3%–174.1%	6.26 (3.235) – 27.87 (24.085)	0.501	0.767

Comments: [1] Numbers are given as range of means (SE); [2] for lower-bound estimate ε; [3] for multiple comparisons.

Table 4. The repeatability coefficients of selected variables, the range of diversity of the coefficient variation of measurement, range of estimation of average measurements for the examined group of people in subsequent repetitions and analysis of the variation of average estimation.

Variable	Q_w	rSD	Range of means estimation[1]	P^2	P^3
Left side of the body					
ac-PF	0.999	0.17%–0.55%	892.21 (43.149) – 893.00 (43.236)	0.808	0.968
ac-IC	0.999	0.38%–0.95%	318.46 (15.732) – 320.08 (15.442)	0.805	0.951
ac-PSIS	0.999	0.36%–0.56%	380.00 (16.621) – 381.33 (16.577)	0.795	0.916
Right side of the body					
ac-PF	0.999	0.15%–0.52%	889.34 (42.258) – 893.00 (41.804)	0.415	0.491
ac-IC	0.994	0.48%–1.40%	322.61 (15.139) – 326.09 (15.447)	0.347	0.335
ac-PSIS	0.994	0.45%–0.98%	377.89 (17.896) – 381.18 (17.088)	0.459	0.530

Comments: [1] Numbers are given as range of means (SE); [2] for lower-bound estimate ε; [3] for multiple comparisons.

viously that the non-invasive basic physiotherapeutic measurements are valid and reliable only when performed by specialized physiotherapist.[39] Thus, the validation process for SpinalMeter diagnostic tool was a necessity. Moreover, the SpinalMeter has been validated and used previously for the assessment of the SKOL-AS treatment effectiveness.[30] Based on the literature, there is quite small number of scientific research checking the specific posture assessment methods.[41,42] Only few of these studies assessed the body posture in sitting position.[43] Thus, in this study the overall validation process of SpinalMeter has been performed during different body positions and quite consistent results were obtained.

5.2. Interpretation

It is well known that the trunk rotation depends on Cobb angle and the apical vertebral rotation (AVR). It was also shown that Cobb angle correlates with both the spinous process angle (SPA) and AVR (correlation coefficients *r* were 0.93 and 0.68, respectively). Moreover, those two factors i.e. SPA and AVR improve the prediction of the Cobb angle.[44] In this study, the transverse plane deformation was analysed by assessing the ATR. Krawczyński et al. (2006) proved that the thoracic curvatures by means of ATR correlate with Cobb angle as well as the longitudinal axis rotation of the apical vertebrae.[45]

The Cobb method is the most commonly used diagnostic tool in the assessment of the angular value of scoliosis (the 'gold standard'). However, this requires an X-ray examination as well as a qualified specialist. It was shown that more experienced specialists could measure the Cobb angle better than less experienced one (spine orthopaedists vs. orthopaedic residents). That demonstrated difficulties in the method, which cannot even be overcome by expertise.[46] It is well known that multiple X-ray examinations pose a risk of adverse outcomes and are not quite safe. Thus, it is advised to limit the number of radiological evaluations.[47] This study was based on the use of clinical measurements using SpinalMeter, which were validated and proposed in order to decrease the children exposure to the additional dose of ionising radiation. The most reliable measurements were assessed for the distance from ac-PF, ac-IC and ac-PSIS for both right and left side of the body. Thus, this could be used for the initial diagnosis when bad posture is diagnosed. Additionally, good repeatability was shown in the case of asymmetry measurements. Some of the distance and asymmetry measurements could be used simultaneously during some control visits rather than radiographic examination. On the other hand, the measurements in the Gibbo position (trunk flexion) – the distance of the transverse process from midline – were perceived as the lowest repeatable measurements and this couldn't be used in the postural assessment.

In conclusion, the biggest advantage of SpinalMeter device is that it is free of radiation emission and it requires no special protection or shielding for either patients or operators. Moreover, the repeated measurements during control visits could be easily performed, so the progression of the pathology and the effectiveness of the treatment protocol and rehabilitation could be determined.

5.3. Limitations

This research presents the analysis based mostly on clinical assessment. This was due to the fact that multiple X-ray exposures for the experiment would be too burdensome for the children. This could not be acceptable for ethical reasons. Thus, the simple method for postural diagnosis has been proposed.

In this study, the Bunnell's scoliometer was used for the determination of the ATR. This could have an impact on the obtained results because of the shortness of the scoliometer arm (8.5 cm). In cases with more lateral crest of the rib hump the measurements might be underestimated.

The advantages of SpinalMeter have been shown. However, the specific limitations have also been observed. Firstly, the development and preparation of the results is based on manual fitting of specific points, which is time-consuming. All markers on the whole body need to be precisely pointed out by a specialist, who should have good palpation skills. Moreover, the correction of fitting is also needed and should be done by an analyst. This could be overcome by some modernizations of SpinalMeter software towards more precise and computerized data analysis.

Moreover, the SpinalMeter does not replace X-ray measurements. It is the complementary diagnostic tool, which could limit the unnecessary imaging of the spine during following control visits. Thus, the monitoring of the patient by SpinalMeter could bring the need for radiographic assessment only when the results from it will deteriorate.

What is more, the SpinalMeter is cheaper than other similar devices as Diers (Germany) and Formetric 4D and could be combined with CervicalMeter and baropodometric platform.

As well as considering how representative the sample is, it is important also to consider the size of the sample. But estimating the sample size for estimation studies requires preliminary research and knowledge of certain measures of variability, established on the basis of previous study of a similar nature or preliminary research, e.g. Common Standard Deviation (root-mean-square standardized effect – RMSSE), expected proportion, expected mean, clinically important difference, or others. There have not been similar studies to our research. In addition, the programs or algorithms (Nquery-Advisor, PASS 2020 Power, Statistica) dedicated to assess the sample size necessary for the research are based only on some analysis schemes that could not be applied in this study.

This study was quite difficult to prepare. Not only the samples size was the limitation but the high amount of images (300 photos) and measurements (1020) which should be analysed by one specialist. This was quite problematic. The amount of work done for manual measure (i.e. the asymmetries etc.) took much time.

6. CONCLUSIONS

This study opens new perspectives in the assessment of posture in girls (e.g. with scoliosis) as it provides the diagnostic tool which does not need specific equipment and is radiation free. SpinalMeter is a promising device, which could be introduced in routine posture assessment and during control visits.

The precise and reliable postural biometrical measurements were performed in the case of the distance between anthropometric points and asymmetry of pelvis and scapula. These measurements could be useful in the assessment of girl's posture when visiting the pediatrician.

Conflict of interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Ethics

All the procedures performed in this study involving human participants conformed to the ethical guidelines of the 1975

Declaration of Helsinki (and its later amendments or comparable ethical standards) as reflected in a priori approval by the institution's human research committee and followed the Adapted Physical Activity (APA) Ethics Standard. The experiment was conducted with the understanding of each subject. All subjects as well as their parents gave written informed consent to participate in this study. The protocol was approved by the Ethics Committee of Stanislaw Popowski Regional Specialized Children's Hospital in Olsztyn, Poland (number of approval: ZE/1/2018/WSSD; date of approval: 10 October 2018). All subjects as well as their parents gave written informed consent to participate in this study.

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