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Plasma level of laminin 5 and collagen IV in cryptorchidism

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ABSTRACT

Purpose: Laminin 5 and collagen IV are the main compounds of the extracellular matrix of the germinal epithelium. The purpose of this study was to evaluate the concentration of these two markers of fibrosis in the plasma of boys with congenital unilateral cryptorchidism.

Materials and methods: The study group comprised 43 boys aged 1–3 years with congenital unilateral cryptorchidism. The control group included 54 healthy, age matched boys, admitted for planned hernioplasty. To assess laminin 5 and collagen IV in the plasma of boys with unilateral cryptorchidism, we used a new biosensor with Surface Plasmon Resonance Imaging technique detection.

Results: The median concentration of laminin 5 and collagen IV in the serum of boys with congenital, unilateral cryptorchidism was higher than in boys with normal scrotal testis. The difference was statistically significant ($p < 0.0001$). We did not notice a correlation between a higher position of the testicles in the inguinal and/or their condition and levels of laminin 5 and collagen IV in the plasma.

Conclusion: Laminin 5 and collagen IV concentrations in the plasma were higher in patients with congenital unilateral cryptorchidism. We believe that in the future, our results could be compared with fertility level in adulthood.

1. Introduction

Cryptorchidism (undescended testis), the most common urogenital congenital defect, is connected with a well-known higher risk of testicular cancer and reduced fertility in adulthood [1]. Therefore, the main purpose of treatment should be to eliminate or reduce the risk of these long-term consequences. It is difficult to predict the potential impact on male fertility, especially when prepubertal boys are operated on. The correct testis position, and lack of testis atrophy after operation, is not equivalent to successful treatment and proper functioning of the male gonads in the future [2].

We are still looking for non-invasive tests to check the status of the germinal epithelium of the undescended testicles. There is no consensus on the role of testicular biopsy in prepubertal boys with cryptorchidism. The internal wall of the germinal epithelium is the basal membrane (BM). Laminin 5 and collagen IV are one of the compounds of the extracellular matrix (ECM) and both are secreted by Sertoli cells [3]. Based on research in patients with varicocele, those cells play an important role in the proper functioning of the testes [3]. They transmit messages from the ECM to the inner compartment.

We do not know the pathophysiology of fibrosis in cryptorchid testicles, but we know that untreated male gonads have a wide range of histopathological changes, such as different degrees of basal membrane thickening, decrease in seminiferous tubule diameter, dystrophic calcification, and Leydig cell hyperplasia [4]. Furthermore, a study in rats found that testicular fibrosis may be prevented with mast cell blocker [5]. The authors observed that fibrous thickening of the wall of the seminiferous tubule started at prepubertal age and was correlated with an increased number of active mast cells. It has been recognized that laminin 5 and collagen IV are in the basal lamina of the basement membrane and that laminin 5 deposit in adult cryptorchid testes formed numerous deep invaginations into the seminiferous epithelium [6].

Therefore, the aim of this study was to evaluate the relationship between congenital unilateral cryptorchidism and basal membrane compounds: laminin 5 and collagen type IV. To measure the plasma levels of laminin 5 and collagen IV, the commercial enzyme-linked immunosorbent assay, the double-antibody sandwich ELISA, could be used [7].

What is worth emphasizing, in this research we used a new, label-free, highly selective biosensor with Surface Plasmon Resonance

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Imaging (SPRI) technique detection. SPRI is a technique to monitor biomolecular interactions. It is an optical-detection technique and allows real-time monitoring of reactions between biomolecules. The combination of SPRI technique with the biosensors is at the forefront of analytical methods of multi-analyte measurements [8]. The SPRI signal is dependent on a change in wavelength and the angle of polarization of light. It is also directly proportional to the mass change on the surface of the metal [9]. The biosensor is usually a gold-coated glass with a layer of active biomolecule as a receptor for a determined compound. SPRI biosensors are based on very specific interactions between enzyme and inhibitor or antigen and antibody. This allows determining the concentration of the analyte directly in the sample [10].

This new test has been already used in different clinical research to determine, e.g. ubiquitin carboxyl-terminal hydrolase 1 (UHCL1), 20S proteasome, immunoproteasome, matrix metalloproteinase-2, laminin 5 with collagen IV and cathepsin D [11–15]. It should be emphasized that the SPRI biosensor is a promising new laboratory technique with a wide spectrum of advantages, e.g. rapid, high-throughput and multiplex detection of protein arrays for proteomics studies, biomarker screening, disease prognosis, and drug discovery. To our knowledge this is the first study assessing the plasma levels of laminin 5 and collagen IV in children with unilateral undescended testicles.

2. Materials and methods

2.1. Patients

We included 43 healthy boys with congenital unilateral cryptorchidism, without any chronic disease aged 1–3 years (mean = 23 months), and 54 generally healthy boys, with both testes in the scrotum, aged 1–3 years (mean = 25 months), who were admitted to elective surgery of the inguinal hernia. It was a prospective study conducted on children from the Department of Pediatric and Urologic Surgery, Medical University of Bialystok (Poland), between 2016-2017. We excluded children with abdominal testis, previous operations, any hormonal treatment, and additional chronic disorders. All testes were in prepubertal stage and sizes. Apart from cryptorchidism, patients were healthy. The plasma samples were taken, and the clinical examination was performed on the day of hospital admission, before the operation. The size and position of the undescended testicles were examined during the surgical procedure.

2.2. Methods

In the morning on the day of surgery 2 ml venous blood samples were taken and collected in EDTA tubes. Subsequently, the blood was centrifuged, and the plasma was frozen at −80 °C.

The plasma levels of laminin 5 and collagen IV were measured using highly selective SPRI biosensors. In order to determine the laminin 5 and collagen IV concentrations, plasma samples were diluted with phosphate-buffered saline (PBS) (1000-fold for laminin 5 determination and 2-fold for collagen IV determination).

2.2.1. Biosensor preparation and SPRI measurements

Biosensors for laminin 5 and collagen type IV determination were manufactured on glass chips as described previously [16,17]. The chip, was immersed in 20 mM of cysteamine ethanolic solutions for a minimum of 2 h and, after rinsing with ethanol and water, dried under a stream of nitrogen. Rabbit polyclonal antibody specific to human laminin 5 (concentration: 5 ng/ml) and monoclonal mouse anti-human collagen type IV (concentration: 6 μg/ml) were used as receptors. The antibody solution (50 μl) was mixed with 250 mL of 50 mM N-hydroxysuccinimide (NHS), 250 mL of 200 mM N-Ethyl-N'-(3-dimethylaminopropyl)carbodiimide (EDC), and 100 mL of carbonate buffer placed on the amine-modified surface, and incubated at 37°C for 1 h. The chip was then treated with the sample solutions (3 μl) for 10 min, rinsed with the buffer and dried, and the SPRI measurement was performed.

SPRI measurements were performed on previously described apparatus [18]. The signal of the light was measured twice based on the registered images, after immobilization of the receptor, and then after interaction with the sample containing the analyte. The SPRI signal, which is proportional to the mass of entrapped laminin 5 or collagen IV, was obtained as the difference between the signals before and after interaction with the analyzed sample. Non-specific binding was eliminated by preparing samples in PBS buffer and by background correction. The concentration of laminin 5 and collagen IV was determined from the calibration curves (prepared before sample measurements). The procedure of the prepared calibration curves was described in previous articles [16,17].

2.3. Ethical issues

The study was approved by the local ethics committee of the Medical University of Bialystok (Poland) (R-I-002//152/2014, R-I-002/178/2009). All parents gave written, informed consent for this study. The study was conducted in accordance with the 1964 Declaration of Helsinki with its later amendments.

2.4. Statistical analysis

Statistical analysis was performed using the STATISTICA PL 12.5 program. All the results of the concentrations are presented as median with 25th and 75th percentiles.

Receiver Operator Characteristic (ROC) curves were constructed to plot sensitivity against specificity of high plasma laminin 5 and collagen IV levels as diagnostic tests for unilateral cryptorchidism. The areas under the ROC curves (AUC) were calculated and compared with the AUC (0.5) of the non-diagnostic test (the line with the slope).

To determine correlation between laminin 5 and collagen IV plasma concentrations, the Spearman test was used.

3. Results

There was no statistically significant difference in the age distribution between the study groups. Most undescended testicles were localized in the superficial inguinal pouch (n = 38) and 5 patients had their testis near the external ring of the inguinal canal. The median concentration of laminin 5 in the blood plasma of boys with cryptorchidism was higher than in the control group (Fig. 1). The difference was statistically significant ($p < 0.0001$). We also noticed a statistically significant difference ($p < 0.0001$) between collagen IV levels in the boys with cryptorchidism and boys with normal male gonads. (Fig. 2). We did not find statistically significant correlations between laminin 5 and collagen IV concentrations. No significant correlation between plasma levels of laminin 5 and collagen IV was found (Spearmen test) ($r = -0.18$).

To evaluate if laminin 5 and collagen IV plasma levels were useful to prove the diagnosis of cryptorchidism, we performed a ROC curve analysis, comparing cryptorchid patients and healthy boys as controls. The AUC for laminin 5 was 0.89 (95% CI 0.829–0.951) (Fig. 3.). The AUC for collagen IV was 0.996 (95% CI 0.988–1) (Fig. 4).

The best cut-off value for collagen IV (129.26 ng/ml) had very high specificity and sensitivity (100% and 97.7%, respectively) to diagnose unilateral cryptorchidism. The best cut-off value for laminin 5 (53.05 ng/ml) had lower than collagen IV specificity and sensitivity (77.4% and 83.7%, respectively) to diagnose unilateral cryptorchidism. (Table 1.).

4. Discussion

Male infertility and a decline in sperm quality have been increasing [19]. Approximately 20% up to 30% of infertility cases are due to the

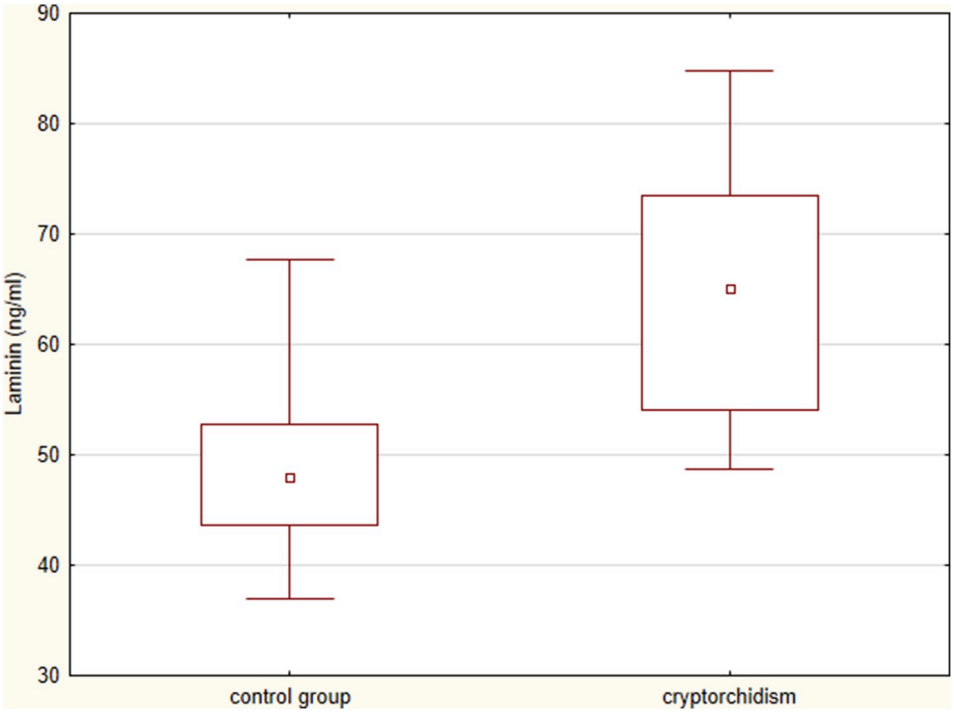

Fig. 1. Plasma laminin 5 concentration in boys with cryptorchidism.

male factor [20]. In heterogeneous causes, cryptorchidism is one of the non-obstructive causes of male infertility and approximately 10% of men have a history of undescended testis [21]. Undoubtedly, the risk of infertility in cryptorchidism is higher in the bilateral form [21,22]. On the contrary, the vast majority of children, suffer from a unilateral defect, and in this group research results are mutually exclusive, partly because studies evaluate different parameters [23].

It is difficult to predict the fertility potential in the future. For example, though the levels of gonadotropin are normal, the number of germ cells could be decreased [24]. Orchiopexy performed in the recommended time (between 6 and 12–18 months of age) [25] does not always guarantee normal germ cell development. Hadziselimovic and Herzog [26] showed in their study that despite a really early surgical correction - before 6 months of age, 35% of boys will be infertile, despite a normal number of germ cells per tubule during testicular biopsy. On the contrary, so far only the results of earlier operations have been studied [27,28]. Fertility has not been compared between early and late age orchiopexy groups yet. However, Park et al. [29] compared

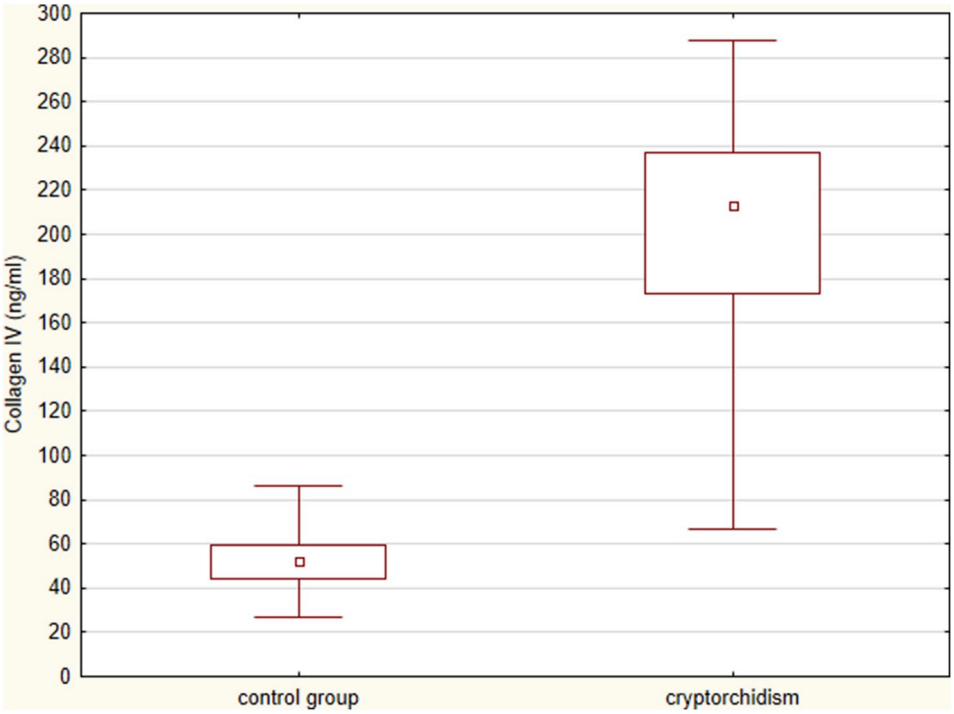

Fig. 2. Plasma collagen IV concentration in boys with cryptorchidism.

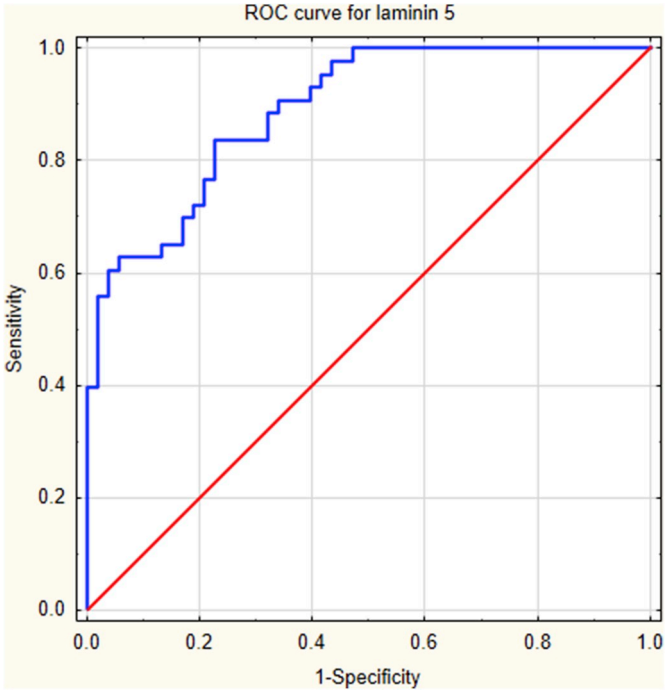

Fig. 3. ROC curves for laminin 5 in plasma as a diagnostic test for unilateral cryptorchidism.

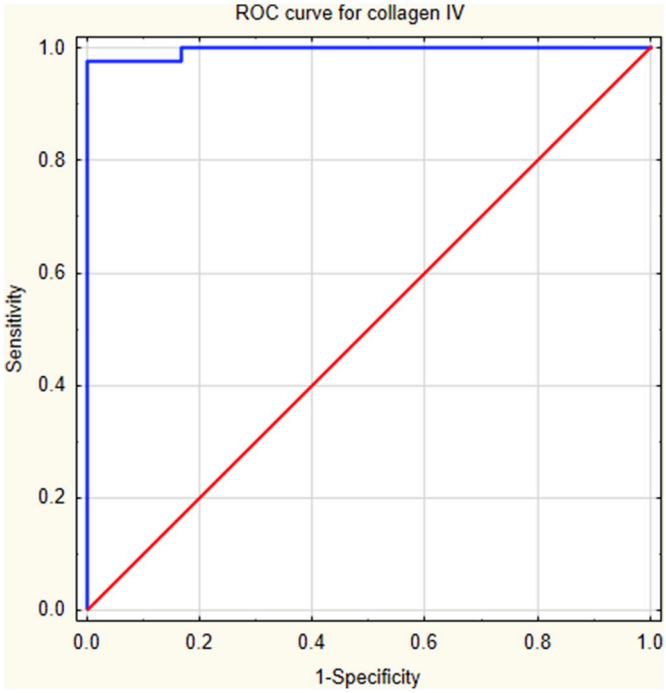

Fig. 4. ROC curves for collagen IV in plasma as a diagnostic test for unilateral cryptorchidism.

histological parameters in boys with unilateral inguinal cryptorchid testes depending on the age. Their results show that as the patient's age increased, the germ cell count decreased, whereas interstitial or peritubular fibrosis increased. Similarly in another study, later age of orchiopexy was associated with reduction of both – the number of Leydig cells and germ cells per tubule. Contrary, histological samples showed improvement in the tubular diameter and reduction of peritubular fibrosis in patients after early operation [29,30].

The last years have brought a new imaging tool: shear wave elastography (SWE), an ultrasound technique to assess soft tissue elasticity and strain [31]. It was first used to check testicular elasticity in 2011 [32]. According to the specific elasticity scores, undescended testicles have been distinguished from reactive lymph nodes [33]. SWE can be used to predict interstitial fibrosis and the severity of histologic damage.

Table 1
Validity indications of plasma laminin 5 and collagen IV levels in prediction of unilateral cryptorchidism.

Parameter	Laminin 5 Plasma	Collagen IV Plasma
Cut-off value	53.05 ng/ml	129.26 ng/ml
Sensitivity	83.7%	97.7%
Specificity	77.4%	100%
PPV	75%	100%
NPV	85.4%	98.2%
Accuracy	80.2%	99%

PPV - positive predictive value; NPV - negative predictive value.

The basal membrane (BM) is a specialized structure of the extracellular matrix. The main components of BM are laminin 5 and collagen IV [34,35], which play a crucial role in the regulation of the biological cellular functions [3]. Collagen IV stabilizes BM, while laminin 5 could bind to collagen IV and epithelial cells [36]. Interestingly, both of these proteins have a direct relationship and strong connection with Leydig cells. On the Leydig cell surface, there are fragments of laminin 5 and collagen IV, although cells are not surrounded by a BM. Additionally, ECM affects the proliferation of those cells, testosterone production, and genes. This suggests that any alteration of ECM can influence the steroidogenic capacity of Leydig cells and steroidogenesis [6]. Interstitial fibrosis is one of the most important histological changes in cryptorchid testis [37].

Plasma laminin 5 and collagen IV concentration is associated with fibrosis of the liver, e.g. in non-alcoholic fatty liver disease [38] or in hepatitis C [39]. These components of ECM are called class I sensitive markers of liver fibrosis [40]. Some authors postulate the replacement of the invasive liver biopsy with the assessment of biomarkers such as laminin 5 and collagen IV [41].

In cryptorchidism, but also in other testis disorders such as varicocele, Sertoli cell-only syndrome (SCO) and Klinefelter syndrome, the BM is thickened due to high deposition of collagen in the extracellular space [42]. The classic histology of undescended testis is usually similar to previous mentioned testis disorders: interstinal fibrosis, disturbances in the count and quality of germ cells, Sertoli and Leydig cells [37]. An experiment on cryptorchid mouse revealed, disruption of the blood-testis-barrier [43]. Histological samples from patients with Klinefelter syndrome presented a natural process of progressive fibrosis and hyalinization and degeneration of the tubules [30], which is almost always associated with inferility [44]. Collagen IV index was significantly correlated with basement membrane thickness [45]. The intraoperative biopsy showed a smaller tubular diameter and lower number of germ cells, which was associated with higher fibrosis grades [46]. Furthermore, in SCO only the localization of laminin 5 and collagen IV are different from healthy testes [47].

In the cryptorchid testis, peritubular or interstitial fibrosis was significantly higher with increasing patient age [29]. Our results showed for the first time that patients suffering from unilateral cryptorchidism have significantly higher plasma levels of laminin 5 and collagen IV. We found that the mean plasma concentration of laminin 5 and collagen IV was significantly higher when compared to the control group ($p < 0.0001$). In young children, as in our control group, the cause of the inguinal hernia is patent processus vaginalis. In contrast to adults, no alteration in collagen subtypes 1:3 ratio have been noticed [48]. In most cases, pediatric patients with inguinal hernia are healthy without connective tissue disorder. Higher laminin 5 and collagen IV concentrations in the plasma of boys with undescended testis may reflect

ECM damage and inform about fibrosis of the testis. Undescended testis may be associated with histological abnormalities, even if early and successful orchiopexy was performed [26]. The higher temperature in the inguinal canal or in the abdominal cavity could damage the temperature-sensitive blood-testis barrier. Even slight elevation of temperature could disrupt spermatogenesis and cause thermal injury of the testis. Benof et al. [49] showed thermal injury of the testicles in cryptorchidism and varicocele. Histological examination of the testicles in adolescents with varicocele showed loss of actin in cells, which are near the basement membrane of the seminiferous tubules. Testicular thermoregulation also does not work properly in patients with cryptorchidism and therefore spermatogenesis may be interrupted. The effect of heat stress on germ cells is apoptosis and autophagy [50]. Furthermore, an increased temperature causes lower sperm counts [51], testicular germinal atrophy, and loss in testicular weight [50]. Besides, disorder of the hypothalamic-pituitary-testicular axis maturation and hence delayed and ineffective transformation of gonocytes into type A dark (Ad) spermatogonia (called 'mini-puberty') [52,53] may impair spermatogenesis in undescended testes [53]. Undescended testes have significant lower RNA levels for spermatogonial and gonocyte marker genes [54] and it is related with disruption of the mini-puberty period and lower level of testosterone. Biopsies from undescended testes showed downregulated positive regulatory domain-containing (PRDM9) genes in tissue gonads which is connected with absence of Ad spermatogonia [55]. PRDM9 encoded histone methyltransferases that are essential for germ cell development. Additionally, in testes with high risk of infertility (lack of Ad spermatogonia) Hadziselimovic et al. [56] found insufficient PROK2, CHD7, FGR1 and SPRY4 gene expression. Deficiency of luteinizing hormone secretion and impairment of the mini-puberty are likely to result from a compromised gene expression.

The goal of cryptorchidism treatment is to prevent testicular heat injury and maintain the function of the testis for future fertility. Semen analysis, sexual hormonal status, and finally paternity rate are used to assess fertility. However, these tests are done mainly in adulthood. Long-term follow up, especially after 18 years of age, is still challenging.

Another option of checking the germinal epithelium of the undescended testis is biopsy. It provides significant information about the germinal epithelium of the testis, but it is still an invasive method. Although some authors suggest that prepubertal testicular biopsy does not damage the undescended male gonads, after biopsy anti-sperm antibodies were not found, and testicular biopsy did not increase the rate of testicular microlithiasis. Still, this procedure is controversial in young children [2].

Therefore, a non-invasive laboratory technique is preferable. We believe that SPRI biosensor could be an alternative technique to invasive testis biopsy. The great advantage of this method is that it is easy, cheap, and reproducible. We can confirm the condition of the reproductive epithelium in the undescended testes and inform parents about possible problems concerning the future fertility of the child. What is worth emphasizing, by means of ROC curve analysis, both proteins (Figs. 3 and 4), especially the measurement of plasma collagen IV level, and to a lesser degree laminin 5 plasma level, were found to be a reliable test for discriminating unilateral cryptorchidism from healthy patients. The positive predictive value (PPV) for collagen IV was interestingly high (100%), and the negative predictive value (NPV) for collagen IV, for the diagnosis capacity in the exclusion of undescended testis versus boys with testis in the scrotum, was likewise high (98.2%). The sensitivity and specificity for collagen IV were 97.7% and 100%, respectively. Laminin 5 and collagen IV concentrations in the plasma of boys with cryptorchidism may reflect damage to the germinal epithelium of the testis. The results of our study may be helpful in assessing the conditions of undescended testis before operation. The higher plasma concentration of laminin 5 and collagen IV, as the main compounds of ECM, could reflect basement membrane disruption or turnover and higher fibrosis in the undescended testis. A significant problem is also to predict testicular function in men with a history of acquired cryptorchidism. There are two options of treatment, one is 'wait and see' protocol [57], because in over half of the cases testes descent before puberty, and the second option is a surgical intervention at diagnosis [58,59]. Research results are contradictory [57,60]. In our opinion, the levels of laminin 5 and collagen IV could be useful to assess the degree of fibrosis in acquired undescended testes or to assess the differences in cryptorchid testis age groups.

5. Conclusions

We found that plasma levels of laminin 5 and collagen IV were higher in patients with undescended testes. We believe that in the future our results could be compared with fertility level in adulthood. Further studies are needed to assess of testicular damage.

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The author contribution

Study Design: Marta Komarowska, Adam Hermanowicz, Ewa Gorodkiewicz.

Data Collection: Marta Komarowska, Adam Hermanowicz, Ewa Matuszczak, Wojciech Debek, Łukasz Oldak, Anna Sankiewicz, Beata Szymańska.

Statistical Analysis: Robert Milewski.

Data Interpretation: Wojciech Debek, Marta Komarowska, Adam Hermanowicz.

Manuscript Preparation: Marta Komarowska, Adam Hermanowicz.

Literature Search: Marta Komarowska, Adam Hermanowicz.

Funds Collection: Adam Hermanowicz.

Declaration of competing interest

The authors declare no conflict of interests.

References

[1] Mathers MJ, Sperling H, Rübben H, Roth S. The undescended testis: diagnosis, treatment and long-term consequences. Dtsch Arztebl Int 2009;106(33):527–32.
[2] Ritzén EM, Bergh A, Bjerknes R, Christiansen P, Cortes D, Haugen SE, et al. Nordic consensus on treatment of undescended testes. Acta Paediatr 2007;96(5):638–43.
[3] Santoro G, Romeo C, Impellizzeri P, Gentile C, Anastasi G, Santoro A. Ultrastructural and immunohistochemical study of basal lamina of the testis in adolescent varicocele. Fertil Steril 2000;73(4):699–705.
[4] Koni A, Ozseker HS, Arpali E, Kilinc E, Dogan HS, Akyol A, et al. Histopathological evaluation of orchiectomy specimens in 51 late postpubertal men with unilateral cryptorchidism. J Urol 2014;192(4):1183–8.
[5] Acikgoz A, Asci R, Aydin O, Çavuş H, Donmez G, Buyukalpelli R. The role of ketotifen in the prevention of testicular damage in rats with experimental unilateral undescended testes. Drug Des Dev Ther 2014;8:2089–97.
[6] Govender D, Sing Y, Chetty R. Sertoli cell nodules in the undescended testis: a histochemical, immunohistochemical, and ultrastructural study of hyaline deposits. J Clin Pathol 2004;57(8):802–6.
[7] Tas F, Bilgin E, Karabulut S, Duranyildiz D. Clinical significance of serum laminin and type-IV collagen levels in cutaneous melanoma patients. Mol Clin Oncol 2016;5(1):195–200.
[8] Helmerhorst E, Chandler DJ, Nussio M, Mamotte CD. Real-time and label-free biosensing of molecular interactions by surface plasmon resonance: a laboratory medicine perspective. Clin Biochem Rev 2012;33(4):161–73.
[9] Steiner G. Surface plasmon resonance imaging. Anal Bioanal Chem 2004;379(3):328–31.
[10] Scarano S, Mascini M, Turner AP, Minunni M. Surface plasmon resonance imaging for affinity-based biosensors. Biosens Bioelectron 2010;25(5):957–66.
[11] Toliczenko-Bernatowicz D, Matuszczak E, Tylicka M, Szymańska B, Komarowska M, Gorodkiewicz E, et al. Overexpression of ubiquitin carboxyl-terminal hydrolase 1 [UCHL1] in boys with cryptorchidism. PLoS One 2018;13(2):e0191806.
[12] Matuszczak E, Tylicka M, Hermanowicz A, Debek W, Sankiewicz A, Gorodkiewicz E. Application of SPR imaging biosensor for the measurement of 20S proteasomes in

blood plasma of children with thermal injury. Ann Clin Lab Sci 2016;46(4):407–11.
[13] Matuszczak E, Sankiewicz A, Debek W, Gorodkiewicz E, Milewski R, Hermanowicz A. Immunoproteasome in the blood plasma of children with acute appendicitis, and its correlation with proteasome and UCHL1 measured by SPR imaging biosensors. Clin Exp Immunol 2018;191(1):125–32.
[14] Weremijewicz A, Matuszczak E, Sankiewicz A, Tylicka M, Komarowska M, Tokarzewicz A, et al. Matrix metalloproteinase-2 and its correlation with basal membrane components laminin-5 and collagen type IV in paediatric burn patients measured with Surface Plasmon Resonance Imaging [SPRI] biosensors. Burns 2018.
[15] Gorodkiewicz E, Guszcz T, Roszkowska-Jakimiec W, Kozłowski R. Cathepsin D serum and urine concentration in superficial and invasive transitional bladder cancer as determined by surface plasmon resonance imaging. Oncol Lett 2014;8(3):1323–7.
[16] Sankiewicz A, Lukaszewski Z, Trojanowska K, Gorodkiewicz E. Determination of collagen type IV by Surface Plasmon Resonance Imaging using a specific biosensor. Anal Biochem 2016;515:40–6.
[17] Sankiewicz A, Romanowicz L, Laudanski P, Zelazowska-Rutkowska B, Puzan B, Cylwik B, et al. SPR imaging biosensor for determination of laminin-5 as a potential cancer marker in biological material. Anal Bioanal Chem 2016;408(19):5269–76.
[18] Anna S, Agnieszka M, Zenon L, Beata P, Ewa G. Methods for 20S immunoproteasome and 20S constitutive proteasome determination based on SPRI biosensors. Cell Mol Bioeng 2017;10(2):174–85.
[19] Durairajanayagam D. Lifestyle causes of male infertility. Arab J Urol 2018;16(1):10–20.
[20] Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol 2015;13:37.
[21] Chung E, Brock GB. Cryptorchidism and its impact on male fertility: a state of art review of current literature. Can Urol Assoc J 2011;5(3):210–4.
[22] Gracia J, Sánchez Zalabardo J, Sánchez García J, García C, Ferrández A. Clinical, physical, sperm and hormonal data in 251 adults operated on for cryptorchidism in childhood. BJU Int 2000;85(9):1100–3.
[23] van Brakel J, Kranse R, de Muinck Keizer-Schrama SM, Hendriks AE, de Jong FH, Bangma CH, et al. Fertility potential in men with a history of congenital undescended testes: a long-term follow-up study. Andrology 2013;1(1):100–8.
[24] Fawzy F, Hussein A, Eid MM, El Kashash AM, Salem HK. Cryptorchidism and fertility. Clin Med Insights Reprod Health 2015;9:39–43.
[25] Ritzén EM. Undescended testes: a consensus on management. Eur J Endocrinol 2008;159(Suppl 1):S87–90.
[26] Hadziselimovic F, Herzog B. The importance of both an early orchidopexy and germ cell maturation for fertility. Lancet 2001;358(9288):1156–7.
[27] Tseng CS, Chiang IN, Hong CH, Lu YC, Hong JH, Chang HC, et al. Advantage of early orchiopexy for undescended testis: analysis of testicular growth percentage ratio in patients with unilateral undescended testicle. Sci Rep 2017;7(1):17476.
[28] Kollin C, Karpe B, Hesser U, Granholm T, Ritzén EM. Surgical treatment of unilaterally undescended testes: testicular growth after randomization to orchiopexy at age 9 months or 3 years. J Urol 2007;178(4 Pt 2):1589–93. discussion 93.
[29] Park KH, Lee JH, Han JJ, Lee SD, Song SY. Histological evidences suggest recommending orchiopexy within the first year of life for children with unilateral inguinal cryptorchid testis. Int J Urol 2007;14(7):616–21.
[30] Tasian GE, Hittelman AB, Kim GE, DiSandro MJ, Baskin LS. Age at orchiopexy and testis palpability predict germ and Leydig cell loss: clinical predictors of adverse histological features of cryptorchidism. J Urol 2009;182(2):704–9.
[31] Ucar AK, Alis D, Samanci C, Aslan M, Habibi HA, Dikici AS, et al. A preliminary study of shear wave elastography for the evaluation of unilateral palpable undescended testes. Eur J Radiol 2017;86:248–51.
[32] Patel K, Sellars ME, Clarke JL, Sidhu PS. Features of testicular epidermoid cysts on contrast-enhanced sonography and real-time tissue elastography. J Ultrasound Med 2012;31(1):115–22.
[33] Agladioglu K, Herek D, Herek O, Agladioglu SY, Cördük N, Ozhan B. Can ultrasound elastography be used as a new technique in the differentiation of undescended testes and reactive lymph nodes in children? Clin Radiol 2015;70(11):1269–75.
[34] Harvey SJ, Perry J, Zheng K, Chen D, Sado Y, Jefferson B, et al. Sequential expression of type IV collagen networks: testis as a model and relevance to spermatogenesis. Am J Pathol 2006;168(5):1587–97.
[35] Siu MK, Cheng CY. Extracellular matrix: recent advances on its role in junction dynamics in the seminiferous epithelium during spermatogenesis. Biol Reprod 2004;71(2):375–91.
[36] Mazaud Guittot S, Vérot A, Odet F, Chauvin MA, le Magueresse-Battistoni B. A comprehensive survey of the laminins and collagens type IV expressed in mouse Leydig cells and their regulation by LH/hCG. Reproduction 2008;135(4):479–88.
[37] Hadziselimovic F. On the descent of the epididymo-testicular unit, cryptorchidism, and prevention of infertility. Basic Clin Androl 2017;27:21.
[38] Santos VN, Leite-Mór MM, Kondo M, Martins JR, Nader H, Lanzoni VP, et al. Serum laminin, type IV collagen and hyaluronan as fibrosis markers in non-alcoholic fatty liver disease. Braz J Med Biol Res 2005;38(5):747–53.
[39] El-Mezayen HA, Habib S, Marzok HF, Saad MH. Diagnostic performance of collagen IV and laminin for the prediction of fibrosis and cirrhosis in chronic hepatitis C patients: a multicenter study. Eur J Gastroenterol Hepatol 2015;27(4):378–85.
[40] Jarcuska P, Janicko M, Veselíny E, Skladaný L. Circulating markers of liver fibrosis progression. Clin Chim Acta 2010;411(15–16):1009–17.
[41] Nallagangula KS, Nagaraj SK, Venkataswamy L, Chandrappa M. Liver fibrosis: a compilation on the biomarkers status and their significance during disease progression. Future Sci OA 2018;4(1). FSO250.
[42] Ooba T, Ishikawa T, Yamaguchi K, Kondo Y, Sakamoto Y, Fujisawa M. Expression and distribution of laminin chains in the testis for patients with azoospermia. J Androl 2008;29(2):147–52.
[43] Chen ZJ, Zhang KS, Ge LC, Liu H, Chen LK, Du J, et al. Signals involved in the effects of bisphenol A [BPA] on proliferation and motility of Leydig cells: a comparative proteomic analysis. Toxicol Res [Camb] 2016;5(6):1573–84.
[44] Davis SM, Rogol AD, Ross JL. Testis development and fertility potential in boys with klinefelter syndrome. Endocrinol Metab Clin N Am 2015;44(4):843–65.
[45] Dobashi M, Fujisawa M, Naito I, Yamazaki T, Okada H, Kamidono S. Distribution of type IV collagen subtypes in human testes and their association with spermatogenesis. Fertil Steril 2003;80(Suppl 2):755–60.
[46] Suskind A, Hayner-Buchan A, Feustel PJ, Kogan BA. Fibrosis correlates with detailed histological analysis of human undescended testes. BJU Int 2008;101(11):1441–5.
[47] Gülkesen KH, Erdoğru T, Sargin CF, Karpuzoğlu G. Expression of extracellular matrix proteins and vimentin in testes of azoospermic man: an immunohistochemical and morphometric study. Asian J Androl 2002;4(1):55–60.
[48] Hosgor M, Karaca I, Ozer E, Suzek D, Ulukus C, Ozdamar A. Do alterations in collagen synthesis play an etiologic role in childhood inguinoscrotal pathologies: an immunohistochemical study. J Pediatr Surg 2004;39(7):1024–9.
[49] Benoff SH, Millan C, Hurley IR, Napolitano B, Marmar JL. Bilateral increased apoptosis and bilateral accumulation of cadmium in infertile men with left varicocele. Hum Reprod 2004;19(3):616–27.
[50] Durairajanayagam D, Agarwal A, Ong C. Causes, effects and molecular mechanisms of testicular heat stress. Reprod Biomed Online 2015;30(1):14–27.
[51] Hjollund NH, Storgaard L, Ernst E, Bonde JP, Olsen J. Impact of diurnal scrotal temperature on semen quality. Reprod Toxicol 2002;16(3):215–21.
[52] Goel P, Rawat JD, Wakhlu A, Kureel SN. Undescended testicle: an update on fertility in cryptorchid men. Indian J Med Res 2015;141(2):163–71.
[53] Hadziselimovic F, Zivkovic D, Bica DT, Emmons LR. The importance of mini-puberty for fertility in cryptorchidism. J Urol 2005;174(4 Pt 2):1536–9. discussion 8-9.
[54] Gegenschatz-Schmid K, Verkauskas G, Demougin P, Bilius V, Dasevicius D, Stadler MB, et al. DMRTC2, PAX7, BRACHYURY/T and TERT are implicated in male germ cell development following curative hormone treatment for cryptorchidism-induced infertility. Genes [Basel] 2017;8(10).
[55] Hadziselimovic F, Cathomas G, Verkauskas G, Dasevicius D, Stadler MB. *PRDM* histone methyltransferase mRNA levels increase in response to curative hormone treatment for cryptorchidism-dependent male infertility. Genes [Basel] 2018;9(8):391. https://doi.org/10.3390/genes9080391.
[56] Hadziselimovic F, Gegenschatz-Schmid K, Verkauskas G, Docampo-Garcia MJ, Demougin P, Bilius V, et al. Gene expression changes underlying idiopathic central hypogonadism in cryptorchidism with defective mini-puberty. Sex Dev 2016;10(3):136–46.
[57] van der Plas EM, van Brakel J, Meij-de Vries A, de Muinck Keizer-Schrama SM, Hazebroek FW, Hack WW, et al. Acquired undescended testes and fertility potential: is orchiopexy at diagnosis better than awaiting spontaneous descent? Andrology 2015;3(4):677–84.
[58] Hutson JM, Balic A, Nation T, Southwell B. Cryptorchidism. Semin Pediatr Surg. 2010;19(3):215–24.
[59] Bonney T, Hutson J, Southwell B, Newgreen D. Update on congenital versus acquired undescended testes: incidence, diagnosis and management. ANZ J Surg 2008;78(11):1010–3.
[60] Hack WW, van der Voort-Doedens LM, Goede J, van Dijk JM, Meijer RW, Sijstermans K. Natural history and long-term testicular growth of acquired undescended testis after spontaneous descent or pubertal orchidopexy. BJU Int 2010;106(7):1052–9.