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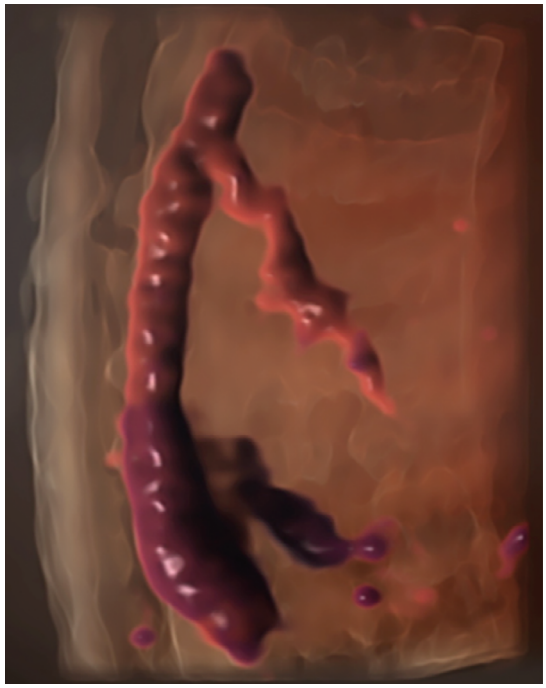

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Hypogonadism — when does genetic diagnosis help in therapy?

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ABSTRACT

Objectives: The objective of the study was to describe phenotype-genotype correlation in selected cases with infertility and emphasise the importance of genetic testing as useful tool for proper treatment decision making

Material and methods: Genetic tests were performed in four patients as a part of diagnostic procedure by Sanger sequencing or targeted next generation sequencing (NGS gene panel).

Results: We found the genetic causes of hypogonadotropic hypogonadism in 3 males and female with infertility.

Conclusions: Genetic testing is carried out when searching for the genetic causes of clinically identified disorders. Genetic diagnostics may also be extremely helpful in treating hypogonadism but requires the assistance of a clinician endocrinologist or andrologist, as well as a geneticist.

Keywords: hypogonadism; infertility; genetic testing

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INTRODUCTION

Infertility is a social problem. More and more couples are having trouble conceiving a child. In addition, more and more elderly women are trying to get pregnant, advanced procreative age means that diagnosis and treatment should be carried out efficiently and effectively.

In many cases, it is possible to diagnose the cause and help the couple become parents. Often, however, the applied treatment does not bring the desired pregnancy.

In people with fertility disorders, various hormonal disorders are often encountered, but their compensation does not result in pregnancy. It turns out that in many cases, Genetic diagnosis is helpful in unexplained infertility, both in women and men with infertility.

In our work, we present the possibilities of using genetic tests to explain the cause of infertility and enable effective treatment.

The first genetic test recommended in the diagnosis of infertility is the assessment of the karyotype. Further genetic testing is ordered depending on the history, physical examination, and abnormalities found. Hormonal tests are very helpful in making further decisions, which allow to direct further genetic diagnostics.

In men with azoospermia, *AZF* and *CFTR* gene mutation testing is indicated. In many cases, however, genetic diagnosis needs to be extended. Different abnormalities should also be expected in patients with hypogonadotropic hypogonadism and different in patients with hypergonadotropic hypogonadism. This also applies to women.

Nowadays, genetic diagnostics in infertility is recommended by many scientific societies [1–6], but it is worth paying attention to the need for cooperation between doctors of various specialties (endocrinologist, geneticist, urologist), as well as educating doctors of various specialties in the field of reproductive health, so that they understand the need to perform specific genetic testing and genetic consultations for couples with infertility.

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Objectives

The objective of the study is to describe phenotype-genotype correlation in selected cases with infertility and emphasise the importance of genetic testing as a useful tool for proper treatment decision making.

MATERIAL AND METHODS

Genetic tests were performed as a part of diagnostic procedure by Sanger sequencing or targeted next generation sequencing (NGS gene panel). The NGS panel included genes such as *SOX10, BMP15, CHD7, DUSP6, FEZF1, FGF17, FGF8, FGFR1, FLRT3, IL17RD, ANOS1, NSMF, PROK2, PROKR2, SEMA3A, SPRY4, WDR11, FSHB, CFTR, AR.*

RESULTS

Male hypogonadotropic hypogonadism — case 1

Male, aged 40, presented for an andrological consultation due to unsuccessfully trying to conceive for around 9 years. Azoospermia was confirmed in repeated semen analyses. The patient has a history of obesity, is post-cholecystectomy, post phimosis surgery, has right kidney stones, and symptoms of hypogonadism (reduced libido, erectile dysfunction), with no signs of hyposmia. On physical examination, a gynoid body type, steatomastia, decreased facial and pubic hair, albinism, a small penis, and reduced testicular volume (as determined by ultrasound, right testicular volume was 5.1 mL, left testicular volume was 5.5 mL — normal volume is 15–25 mL per testes [7]) were found. Magnetic resonance imaging (MRI) of the pituitary gland showed heterogenous contrast enhancement of the anterior lobe of the pituitary gland. Hormone tests revealed hypogonadotropic hypogonadism with normal function of the remaining axes: adrenal, thyroid, and normal prolactin levels. Due to the presence of symptoms of hypogonadism and reduced sexual performance, symptomatic treatments were given, that is, testosterone replacement therapy (Tab. 1).

Table 1. Hormone test results of patient No. 1 with hypogonadotropic hypogonadism and infertility

Parameter	Unit	Result	Normal range
FSH	mIU/mL	0.3	1.5–12.4
LH	mIU/mL	< 0.1	1.7–8.6
Testosterone	nmol/L	5.64	8.64–29
Estradiol	pg/mL	13.5	25.8–60.7
Inhibin B	pg/mL	36	25–325
TSH	uIU/mL	2.0	0.4–4.0
fT4	pmol/L	15.1	12–22
fT3	3.1–6.8	4.31	3.1–6.8
ACTH	pg/mL	25.2	6–56
Cortisol	ug/dL	13.9	2.3–23.3
Growth hormone	uIU/mL	1.2	0.2–10.0
Prolactin	ng/mL	11	5–19

FSH — follicle stimulating hormone; LH — luteinising hormone; TSH — thyroid stimulating hormone; ACTH — adrenocorticotropic hormone

The patient took the recommended testosterone preparation at a dose of 250 mg every two weeks intramuscularly for 8 years. Subsequent semen analyses consistently showed azoospermia.

The patient did not consent to sperm retrieval by a testicular biopsy. Genetic test revealed a hemizygous pathogenic variant of p.Pro366GlnfsTer12 (c. c.1097delC) in *ANOS1* gene (X-linked recessive inheritance pattern) . This is a very rare, pathogenic variant previously described in an Asian patient with Kallmann Syndrome [7, 9, 15].

Testosterone replacement therapy was discontinued, hormonal stimulation of spermatogenesis was implemented using human chorionic gonadotropic (hCG) 5000 units biweekly and follicle stimulating hormone (FSH) 2 × 150 units weekly, obtaining an improvement of spermatogenesis. The first spermatozoa appeared in the semen after 3 months of treatment, after a subsequent three months, the number of spermatozoa in the ejaculate was 21 million (normal ≥ 39), and after yet another three months, the sperm count in the ejaculate came to 97 million. The spermatogenesis cycle from the spermatogonium through to the spermatozoa lasts 74 days; the effects of the stimulation cannot be achieved any quicker. The treatment took longer in this patient as we were dealing with pre-puberty hypogonadism. Note that even such a small (pre-puberty) testicular volume is not a contraindication for the hormonal stimulation of spermatogenesis. Full spermatogenesis was successfully restored in the patient despite the small testicular volume and very low inhibin B concentration.

Female hypogonadotropic hypogonadism — case 2

Female patient, aged 31, presenting at a fertility clinic with unsuccessful attempts to conceive for approximately 2 years. Thus far, treated by various physicians for menses disorders (amenorrhea in the absence of medication). Hormone tests showed hypogonadotropic hypogonadism. Various kinds of hormone treatment were applied (substitution of estradiol progesterone, dydrogesterone, hormone replacement therapy, and ovulation induction with clomiphene, letrozole, and gonadotropins). Ovulation was successfully induced, and ova were collected during *in vitro* fertilisation procedures but the embryo did not develop further and no embryo transfer attempts to the uterine cavity were possible. The patient did not consent to an *in vitro* fertilization (IVF) procedure with a donor's ova. An ultrasound scan revealed a normal image of the reproductive

organ, patent fallopian tubes, and no suspicion of endometriosis was raised. There were no abnormal findings on physical examination and the body mass index was 22.9. The patient was taking 75 microgram per day levothyroxine to treat hypothyroidism.

During the diagnostic tests at the fertility clinic, attention was drawn to the short stature of the patient (measuring 159 cm with a weight of 58 kg). Further diagnostic tests were ordered along these lines (Tab. 2).

Apart from the deficiency of the hypothalamic-pituitary-gonadal axis, a growth hormone deficiency [below the normal ranges for age- and sex-specific serum insulin-like growth factor-1 (IGF-1) levels] was found and confirmed in functional tests. Adrenal insufficiency was also identified [low dehydroepiandrosterone sulphate (DHEAS) concentration and low adrenal reserve in the scope of cortisol secretion]. The DHEAS hormone is essential for the proper maturation of the ovarian follicles.

Genetic testing was carried out and revealed a pathogenic variant in the *PROP1* gene. The patient was qualified for the growth hormone treatment programme in adults. Growth hormone is essential for the growth and maturation of the ovarian follicles. Gonadotropins cannot act normally without the concomitant action of the growth hormone. Growth hormone is key to maintaining normal fertility in women.

Table 2. Hormone test results of Patient No. 2 with combined pituitary hormone deficiency

Parameter	Unit	Result	Normal range
FSH	mIU/mL	0.8	1.5–12.4
LH	mIU/mL	0.7	1.7–8.6
Estradiol	pg/mL	< 10	Cycle phase--dependent
Testosterone	ng/dL	17	13–53
Androstendion	ng/mL	1.7	0.3–3.3
ACTH	pg/mL	5.7	5–45
DHEAS	ug/dL	59	74–410
Cortisol	ug/dL	7.9	7–15
Prolactin	ng/mL	7	5–25
SHBG	nmol/L	20.2	19–155
AMH	ng/mL	11.1	1.2–5.0
IGF-1	ng/dL	56	159–478

FSH — follicle stimulating hormone; LH — luteinising hormone; ACTH — adrenocorticotropic hormone; DHEAS — dehydroepiandrosterone sulphate; SHBG — sex hormone binding globulin; AMH — anti-Müllerian hormone; IGF-1 — insulin-like growth factor-1

Male hypogonadotropic hypogonadism — case 3

Male, aged 37, presented for an andrological consultation due to unsuccessfully trying to conceive for around 6 years. Azoospermia was confirmed in repeated semen analyses. The hormone test results pointed to hypogonadotropic hypogonadism (Tab. 3).

Table 3. Hormone test results of patient No. 3 with hypogonadotropic hypogonadism and infertility

Parameter	Patient's result	Normal range	Unit
FSH	1.3	1.5–12.4	mIU/mL
LH	0.7	1.7–8.6	mIU/mL
Testosterone	1.2	8.64–29	nmol/L
TSH	2.2	0.4–4.0	uIU/mL
ACTH	22	6–56	pg/mL
DHEAS	234	189–523	ug/dL
Cortisol	12.5	7–15	ug/dL
Prolactin	9.4	5–19	ng/mL

FSH — follicle stimulating hormone; LH — luteinising hormone; TSH — thyroid stimulating hormone; ACTH — adrenocorticotropic hormone; DHEAS — dehydroepiandrosterone sulphate

On physical examination, several characteristics of hypogonadism were found almost complete absence of facial hair and gynoid body type. The testicular volume as determined by ultrasound was very small: right testicle 4.6 mL, left testicle 5.6 mL. The patient has been treated with testosterone since adolescence. An MRI of the pituitary gland showed a normal pituitary gland image and normal olfactory bulb. Gonadotropin therapy was implemented [FSH 75 units biweekly, luteinising hormone (LH) 2500 units biweekly]. The testosterone concentration increased above normal levels with higher doses, inhibiting the release of gonadotropin-releasing hormone (GnRH) and, in effect, LH and FSH secretion. Gonadotropin therapy was implemented for a year, but no sperm appeared in the semen.

Genetic test revealed a presence of a rare pathogenic variant p.Glu84GlyfsTer26 in *FGFR1* This was an indication to continue therapy since patients with a *FGFR1* mutation require prolonged gonadotropin therapy (1–2 years) for the activation of spermatogenesis. The therapy turned out to be effective and an ejaculate sperm count of 16.5 million was obtained following 12 months of treatment.

Male hypergonadotropic hypogonadism — case 4

Male, aged 34, presented for an andrological consultation due to unsuccessfully trying to conceive for around 3 years. Healthy until the present, no history of chronic diseases, taking various supplements to improve sperm motility and morphology. As a result of repeated semen analyses, oligoasthenotaratozoospermia syndrome was detected: a reduced number of spermatozoa, lower motility, mostly abnormal sperm morphology, as well as increased seminal fluid viscosity (Tab. 4).

Table 4. Selected semen analysis parameters in patient No. 4 with infertility

Parameter	Patient's result	Reference value
Liquefaction time	15 min	< 60 min
pH	7.8	≥ 7.2
Viscosity	+++	+
Sperm concentration [mln/mL]	8	≥ 15
Total no. of sperm in ejaculate [mln/ejaculate]	41.6	≥ 39
Progressive motility [%]	1	≥ 32
Sperm with normal morphology [%]	1	≥ 4

In line with the andrology guidelines, the patient's medical history was taken, and a physical examination was performed and no abnormalities or deviations from the norm were found. Further diagnostic tests were ordered: a scrotum ultrasound, hormone tests, and semen microbiology analysis. The presence of the following pathogens was confirmed in semen culture: *Enterococcus faecalis* in titres of > 10E5/mL, Gram-positive cocci in titres of 10E3/mL under aerobic and anaerobic conditions, *Staphylococcus*, coagulase negative, in titres of 10E3 under aerobic conditions, and *Mycoplasma hominis* in titres of > 10E4/mL.

The presence especially of the latter pathogen explained the seminogram abnormalities found. It is evident that urogenital infections may lead to male infertility [8]. Mycoplasma hominis is a bacterial infection in men that may cause urinary tract infections, prostatitis, epididymitis, poor semen quality and, ultimately, infertility. This is a sexually transmitted infection and requires concurrent treatment of the partner. The infection may be completely asymptomatic and, with time, may also lead to urinary tract infections, vaginitis, pelvic inflammatory disease, and fallopian tube infertility problems in women. Even an asymptomatic *M. hominis* infection induces a pro-inflammatory immune response in the endometrium and prevents the embryo from implanting within IVF procedures.

Having identified the pathogen, the patient could be treated causally. Antibiotic therapy was implemented in both partners, according to the antibiogram results (ofloxacin and metronidazole in the man, and doxycycline in the woman).

An ultrasound of the scrotum revealed a normal testicular volume but closer to the lower limit of the normal range (right testicle 16.7 mL, left testicle 18.9 mL — with a normal volume for a healthy man ranging between 15–25 mL per testes), grade 1 and 2 varioceles, and right-sided epididymal cysts up to 3 mm.

Table 5. Selected hormone tests in patient No.4 with infertility

Parameter	Patient's result	Normal range	Unit
FSH	14	0.95–11.95	IU/L
LH	7.5	0.57–12.07	IU/L
Estradiol	28.4	< 44	pg/mL
Testosterone	237	240–870	ng/dL
TSH	1.8	0.4–4.4	mIU/mL
Prolactin	12.9	3.4–19.4	ng/dL

FSH — follicle stimulating hormone; LH — luteinising hormone; TSH — thyroid stimulating hormone

Hormone tests showed hypergonadotropic hypogonadism [FSH, LH, testosterone, prolactin, thyroid stimulating hormone (TSH), estradiol, and vitamin D3] (Tab. 5).

There were no pathogens at a follow-up smear test of the partner after doxycycline treatment. However, a urogenital infection was once again detected in the man, this time *Klebsiella pneumoniae* and *Escherichia coli* was present in the semen culture. Antibiotic therapy was once again administered but the diagnostics were extended to include internal medicine causes of recurrent infections like, for example, diabetes. Within the clinical study, the patient was referred for genetic diagnostic testing. The p.Ser1235Arg variant in one allele of the cystic fibrosis *(CFTR)* gene was found. This variant is described in the Human Gene Mutation Database (HGMD) and ClinVar databases as a variant of uncertain clinical significance. The patient's partner was also referred for genetic testing and there was no pathogenic variant of the *CFTR* gene.

Antibiotic therapy was once again given but this time with mucus thinners (like in cystic fibrosis): acetylcysteine, ambroxol, and vitamins, obtaining normal sperm liquefaction and pregnancy in his partner after 2 months.

DISCUSSION

Carrying out genetic testing in patients with endocrinopathies and infertility brings specific advantages. The guidelines of scientific societies recommend [6, 7, 12, 13]:

— carrying out a karyotype test for all men with infertility who have a sperm count of under 10 million sperm per mL;
— additional diagnostics for azoospermia factor (AZF) and cystic fibrosis (CFTR) gene mutation in men with azoospermia.

When diagnosing and treating patients with infertility, we often refer them for additional genetic tests in the search for the causes of endocrine disorders with the hope that we will be able to implement effective treatment programs.

Based on the cases presented above, we are substantiating the rationale for carrying out additional genetic tests.

In order to minimise the costs and increase the chances of finding the genetic causes, the most purposeful seems to be ordering genetic testing depending on the type of identified endocrine disorder(s).

Lessons from case 1

A genetic test explained the cause of azoospermia. A pathogenic variant in *ANOS1* gene caused hypogonadotropic hypogonadism. It is due to this pathogenic variant that gonadotropins FSH and LH are not being secreted by the pituitary gland, which stimulate the testes to produce sperm and testosterone. FSH stimulates Sertoli cells to produce sperm in seminiferous tubules, and LH causes the Leydig cells of the testes to produce testosterone, also conditioning erection. Normal hormone test results give information on spermatogenic activity: the concentration of gonadotropin FSH and LH as well as inhibin B. The absence of FSH secretion by the pituitary gland causes the absence of stimulation of the receptors for FSH in the seminiferous tubules and the absence of spermatogenesis. The absence of LH secretion by the pituitary gland leads to the absence of stimulation of the receptors for LH and disrupts the production of testosterone by Leydig cells. The concentration of inhibin B is considered a marker of spermatogenesis activity in the seminiferous tubules of the testes.

If the testicular tissue is healthy (*e.g.*, not affected by a neoplastic process), hormonal stimulation of spermatogenesis is a highly effective treatment in hypogonadotropic hypogonadism. Even very low concentrations of FSH, LH, testosterone or inhibin B and a small testicular volume do not disqualify a patient when it comes to the possibilities of recreating spermatogenesis, even if the hypogonadotropic hypogonadism is genetically conditioned (*ANOS1* gene pathogenic variant).

The hormone therapy requires 6–9 months for the effects to be seen if the patient never received gonadotropin treatment earlier, although the first spermatozoa may appear as soon as after 3 months of treatment.

Lessons from case 2

Gene *PROP1* (prophet of Pit1) is specific to the pituitary gland. It is expressed in all progenitor cells of the glandular part of the pituitary gland and its mutations give no other clinical symptoms apart from the effects of anterior pituitary lobe hormone deficiency.

Growth hormone is required for ovarian follicular growth and maturation [10]. It has been demonstrated in an animal model that the ovaries are approximately 40% smaller in species without the growth hormone but exhibit all types of follicles (preantral, and small and large antral). Growth hormone deficiency impairs fertility: the ovarian response to treatment with exogenous gonadotropin is 3 times poorer, where a reduced frequency of ovulation is essentially caused by growth hormone deficiency and not by pituitary gonadotropin deficiency. The number of mature ovarian follicles is significantly reduced, although all categories of follicles are represented. The LH-, FSH- and IGF-I-binding capacity of the follicles is not reduced. Growth hormone receptors in women are located in the ovaries on granulosa cells and germ cells, as well as on mammary glands, the uterus, fallopian tubes, and the skin.

Our patient was treated with growth hormone from childhood until the age of 16 years, thanks to which secondary sex characteristics developed normally and there was normal development of the uterus and genital tract. The treatment was later completed because the patient achieved the target height and closure of the epiphyseal cartilages was obtained. However, for the ovarian follicles to normally grow and mature, growth hormone is also required asides from sex hormones. At present, the patient is once again receiving growth hormone treatment within the growth hormone treatment programme in adults with a deficiency of this hormone. She is also taking dehydroepiandrosterone (an adrenal cortex hormone), which is also required for normal ovarian follicle growth.

An abnormal growth and absence of ovarian follicle maturation leads to reproductive failure even during IVF procedures.

Carrying out NGS analysis and identifying a PROP1 mutation in this patient explained the aetiology of the detected disorders. Determination of IGF-1 levels in adult female patients with a short stature and infertility is also advisable. In patients with combined pituitary hormone deficiency, mutations in the *PROP1* gene should also be designated.

Lessons from case 3

With reference to literature reports [3], a pathogenic variant in the *FGFR1* gene causes congenital hypogonadotropic hypogonadism with secondary testicular deficiency. Congenital hypogonadotropic hypogonadism, in light of the *FGFR1* gene pathogenic variant, causes central nervous system developmental disorders in the form of GnRH neuronal migration disruptions, which results in disrupted GnRH production or action. This pathogenic variant has a prevalence in the range of 1/5000 to 1/8000 for men. It is manifest by delayed sexual maturation and infertility. If the man is planning to father children, pulsed administration of GnRH or gonadotropin treatment should be introduced to stimulate sperm production in the testicle.

Patients with a pathogenic variant in *FGFR1* require a longer duration of gonadotropin treatment to stimulate spermatogenesis. In a study by Li et al. [9], a sperm count of more than 20 million after one year of treatment was not obtained in any patient, but after 2 years of gonadotropin

treatment, the sperm count exceeded 20 million in 25.9% of the patients. A longer treatment duration was necessary if the initial testicular volume was below 5 mL and if there was a history of cryptorchidism. The likelihood of the achievement of successful spermatogenesis in patients with a *FGFR1* pathogenic variant is good, but the stimulation may last even up to 2 years. The success rate of achieving effective spermatogenesis after one year of patient stimulation was 35.7%, and 75.9 % after 2 years of treatment. The median duration of achieving the first spermatozoa was 16 months.

Carrying out NGS in this patient with infertility provided information on the fact that the azoospermia was caused by p.Glu84GlyfsTer26 *FGFR1* pathogenic variant. Knowledge on the fact that this pathogenic variant requires prolonged hormonal stimulation of spermatogenesis allowed us to make the right treatment decisions. The decision was made to continue the costly treatment, which turned out to be successful.

It should be noted that the recommendations of the European Academy highlight that in some patients with prepuberty hypogonadism a longer duration of gonadotropin or GnRH treatment may be required (1–2 years).

Lessons from case 4

Pathogenic variant of the *CFTR* gene may cause congenital bilateral absence of the vas deferens (CBAVD) by changing the coding of the CFTR protein, which acts as an ion transporter, regulating the viscosity of the mucus. The numerous CFTR pathogenic variants that have been identified have varied effects on the quality and quantity of the created protein, which leads to a varied spectrum of symptoms ranging from cystic fibrosis (bronchitis and pancreatitis) to oligozoospermia, asthenozoospermia, epididymal obstruction, CBAVD, idiopathic ejaculatory duct obstruction, and infertility [9].

Other properties of seminal fluid of patients with cystic fibrosis have been described in literature, such as: higher viscosity, longer liquefaction time, and a growing incidence of leukocytospermia and pyospermia. Treatment in such cases consists of introducing medication that aids semen liquefaction (acetylcysteine, bromhexine, and guaifenesin) and, if necessary, antibiotics. The use of these medications in the treatment of our patients improved sperm motility and led to a successful pregnancy outcome in his partner. The child was born healthy, with normal screening test results for cystic fibrosis.

The current andrology guidelines recommend testing for *CFTR* gene pathogenic variants only in the case of azoospermia [11]. It seems, however, that testing for *CFTR* gene pathogenic variants should not only be considered in the case of azoospermia but also in other clinically relevant cases. Perhaps CFTR modulator treatment will be available to such patients in the future. At present, dornase alfa can be used which, according to the licensed indications of this drug, can also be used to treat primary ciliary dyskinesia.

The CFTR-related disease suspected due to the oligoasthenoteratozoospermia and higher sperm viscosity was not confirmed in the patient. A p.Ser1235Arg variant in one allele of the CFTR gene was identified in a molecular diagnostic test, which is indicative of being a carrier .

The clinical status of the p.Ser1235Arg variant is controversial. It is described in the Human Gene Mutation Database (HGMD) and ClinVar databases as a variant of uncertain clinical significance. According to CFTR2 database, most individuals with this variant (combined with another CF--causing variant) will be healthy, however, a small number of individuals may develop mild symptoms or be diagnosed with a CFTR-related disorder (CFTR-RD), but symptoms are not expected to be severe enough to meet the definition of CF. Recent literature data indicate that p.Ser1235Arg is not a CF or CBAVD causing variant and should be considered as benign variant , however, a partial penetrance of this variant cannot be ruled out [14].

Interestingly in our patient, the sperm quality improved and, subsequently, a pregnancy was achieved in his partner after implementing treatment used in cystic fibrosis that liquefies mucus, suggesting that this may indeed by a CFTR-related disease and that there are indications to continue looking for another variant of the CFTR gene. Unfortunately, due to lack of DNA of the patient we could not preform extended analysis of CFTR gene. Thus, limitation of our study is that we did not exclude the presence of other *CFTR* pathogenic variants in cis/trans with p.Ser1235Arg in non-screed regions of *CFTR*. Such extended analysis should be always performed in cases with p.Ser1235Arg variant found in *CFTR* gene.

The other general remark is that in case of detecting a pathogenic variant of CFTR gene in the patient, it is recommended to carry out carrier screening for this gene for his/ /her partner. This will allow genetic counselling to be offered to patients who have a chance of having offspring with cystic fibrosis or CFTR-related disorders.

Summary

Genetic testing can be highly useful in diagnosing and treating infertility and the spectrum of tests currently available is becoming wider and wider. Based on the presented cases, we would like to emphasise how important it is to expand the basic genetic testing options offered for couples with infertility available to date (karyotype and *CFTR* gene analysis) to other genes associated with infertility by targeted sequencing panels or whole exome sequencing (WES) for unsolved cases. However, it is important to note that the greatest chances for a proper diagnosis based on

genetic tests are when there are reasonable grounds to suspect a disease based on detailed clinical data. This is because the classification of genetic variants into categories such as benign, pathogenic, likely pathogenic or variants of uncertain clinical significance) should be always made with correlation to clinical symptoms. If the clinical data are lucking, the interpretation of a genetic result is very difficult or in some cases even not possible. Hence, the cooperation of clinicians (endocrinologists and andrologists) with geneticists and clinical geneticists is so crucial. It would then be possible to tailor a genetic testing package depending on the identified disorders and hormone test results.

CONCLUSIONS

Even an extremely small volume of the testes does not rule out the chances of stimulating spermatogenesis if azoospermia is caused by hypogonadotropic hypogonadism with a pathogenic variant of the ANOS1 gene (patient 1).

Sometimes hormonal stimulation of spermatogenesis with gonadotrophins must be continued for longer — even up to 12–24 months (as in the case of the FGFR1 gene mutation in patient 3).

The detection of the PROP1 gene mutation was an indication for treatment with growth hormone, the action of which is necessary for the growth of ovarian follicles (patient 2).

Sometimes, clarification of the causes of recurrent urogenital infections is crucial for effective treatment.

Effective treatment our patients would not be possible without genetic diagnosis. We need understand the need for genetic consultations for couples with infertility. Mutual education of physicians of various specialties dealing with infertile couples is also necessary.

Article information and declarations

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Conflict of interest

The authors declare no conflict of interest.

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Expression of B7–H4 in endometrial cancer and its impact on patients' prognosis

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ABSTRACT

Objectives: The aim of the study was to evaluate the B7–H4 expression in endometrial cancer cells and to investigate its relationship with patient prognosis and clinicopathological features of the disease.

Material and methods: We performed a single-center, retrospective cohort study that included endometrial cancer patients treated between 2012 and 2019. B7–H4 expression in specimens obtained from 63 patients was examined by immunohistochemical staining. The evaluation of B7H4 immunoreactivity was assessed using Immunoreactivity Scoring (IRS) system.

Results: B7–H4 reactivity was observed in all, except one, endometrial cancer patients (98%). Staining intensity: no reaction in one case, weak in 16 (24%) patients, moderate in 25 (37%), and strong in 22 (35%). Twenty-nine (46%) patients showed B7–H4 immunoreactivity in more than 60% of cells, while, in 18 (29%) cases and 16 (25%) patients, the percentages were 30–60% and < 30% respectively. Median IRS was 2 (range 0–6). We found a significantly worse overall survival (OS) rate for patients with high versus low B7–H4 IRS ($p = 0.03$), however, in multivariate analysis, the difference in patient survival was close to the significance level ($p = 0.052$). B7–H4 expression was not related to histopathological type of the tumor, tumor grade, lymph node metastases, or the FIGO stage of the disease.

Conclusions: Our result suggests that B7–H4 expression might be a useful prognostic factor in endometrial cancer.

Keywords: B7–H4; endometrial cancer; IRS; overall survival; immunohistochemistry

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INTRODUCTION

Endometrial cancer (EC) is the sixth most diagnosed cancer in women worldwide. Its highest incidence rate is observed in North America and Europe and the highest mortality rate is reported in Eastern Europe [1].The primary management of EC consists of surgery and/or radiotherapy, as well as systemic therapy in selected cases. Organising patients into prognostic risk groups is crucial for optimal treatment. Prognostic factors in EC are as follows: histopathological subtype, tumor grade, myometrial and cervical involvement, the presence of lymph vascular space invasion (LVSI), and the presence of lymph node or distant metastases. However, thanks to molecular profiling of endometrial cancer, more accurate predictions of patient prognosis can be achieved. Using The Cancer Genome Atlas (TCGA), four molecular subtypes [POLE ultra mutated, microsatellite instability (MSI), copy-number low, and copy-number high] of endometrial cancer were distinguished [2]. For clinical purposes, molecular profiling of endometrial cancer can be simplified and conducted by combining immunohistochemistry analysis and one molecular test (mutation analysis of exonuclease domain of POLE) [3]. However, despite the incorporation of molecular studies, the evaluation of a patient's prognosis is not always clear. This is especially in cases of MSI and copy-number low (non-specific molecular profile; NSMP) where subtypes are not always predictable

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[4]. Therefore, new prognostic factors in endometrial cancer are needed.

Cancerogenesis is an extremely complicated process involving many molecular and cellular mechanisms. A tumor's microenvironment influences the growth and invasion of cancer cells. The T-cells, macrophages, and natural killer cells (NK-cells) are believed to constitute a protective anti-cancer barrier and may play an important role in the antitumor immune response. However, growing tumor cells often develop a variety of mechanisms that help them escape such attention. One of these is a change in the expression of costimulatory molecules, for example members of the B7 family, resulting in impaired recognition of these cells by the host immune system [5]. B7–H4, also known as B7x, B7S1, and VTCN1, is a member of the B7 family, which downregulates the T-cell mediated response. It is responsible for activation and proliferation of T-cells, and cytokine production, and may promote tumor growth. This was discovered in 2003 and since then has attracted the attention of many researchers due to its potential role in human diseases [6, 7]. Although the presence of B7–H4 mRNA is common in peripheral tissues, B7–H4 protein is undetectable, or expressed at low levels in normal somatic tissues, while it is usually overexpressed in inflamed tissue, viral infections, pregnancy and various types of tumor cells [6–8]. B7–H4 also occurs in a soluble form that can be detected in patients' serum [8].

In a normal endometrium, the close interaction of immune cells and hormonal changes results in the proper performance of its various functions. Obesity, hyperestrogenism and advance age, which are related to increased risk of endometrial cancer, have immunomodulatory potential. Endometrial immune cell infiltration does not only change during the menstrual cycle, but also in uterine tumors, and this phenomenon has its clinical implications [9]. Previous studies revealed that a host's immune response might be considered as an independent prognostic factor for patient survival in endometrial cancer [10]. For example, the overexpression of B7–H4 in tumor cells has been investigated as a poor prognostic factor in many neoplasms, such as cervical cancer, breast cancer, colorectal cancer, cholangiocarcinoma, and osteosarcoma [11–15]. The effects of the B7–H4 protein on the histopathological parameters and prognosis of patients with endometrial cancer are not fully understood.

Objectives

In the current study we report on the impact of B7–H4 expression in endometrial cancer cells on patients' clinicopathological parameters and survival.

MATERIAL AND METHODS

Patients

We performed a single center, retrospective cohort study that included endometrial cancer patients treated in the 2nd Department of Obstetrics and Gynecology, Centre of Postgraduate Medical Education, Warsaw, Poland between 2012 and 2019. We included only patients who underwent surgical treatment composed of hysterectomy, bilateral salpingoophorectomy and pelvic lymphadenectomy. Omentectomy was performed when serous endometrial adenocarcinoma was diagnosed. We excluded patients with distant metastases (M1), neoadjuvant chemo- or radiotherapy, patients without lymphadenectomy, and patients who also had paraaortic lymphadenectomy. Following surgical treatment, the included patients underwent adjuvant therapy according to the risk of recurrence and death. In general, low risk patients were observed, intermediate risk patients received vaginal brachytherapy, intermediate-high risk patients received pelvic radiation, and high-risk patients were treated with a combination of both radiotherapy and chemotherapy.

The study was approved by Ethical Committee of Centre of Postgraduate Medical Education, Warsaw (84/PB/2020). Information on any patients who died was retrieved from the database of the Poland National Health System of Poland. Overall survival was calculated from the date of surgery to the date of death or the last follow-up.

Immunohistochemistry

Immunohistochemistry (IHC) analyses were performed using Anti-B7H4 antibody (ABCAM). All IHC studies were performed on 4 μm-thick sections taken from cancerous tumors fixed in 4% buffered-formalin and embedded in paraffin blocks. The specimens for IHC staining were selected according to routine histopathological protocols. Thus, among multiple tumor sections evaluated in haematoxylin and eosin (H&E) stain we selected the most representative specimen with the highest tumor volume and without necrosis. Representative images are presented in Figure 1. Evaluation of B7–H4 immunoreactivity was conducted using the Immunoreactivity Scoring system (IRS). Briefly, the evaluation included the simultaneous assessment of the number of B7–H4-positive cells and the intensity of the immunoreactivity. Staining intensity (A) was evaluated as negative or weak (0), moderate (1) and strong (2). The percentage of stained cells (B) was evaluated using the subjective method of the succeeding approximations, and the results were categorized as: 0 = no immunoreactivity or < 10% of labelled cells; 1 = 11–30% of

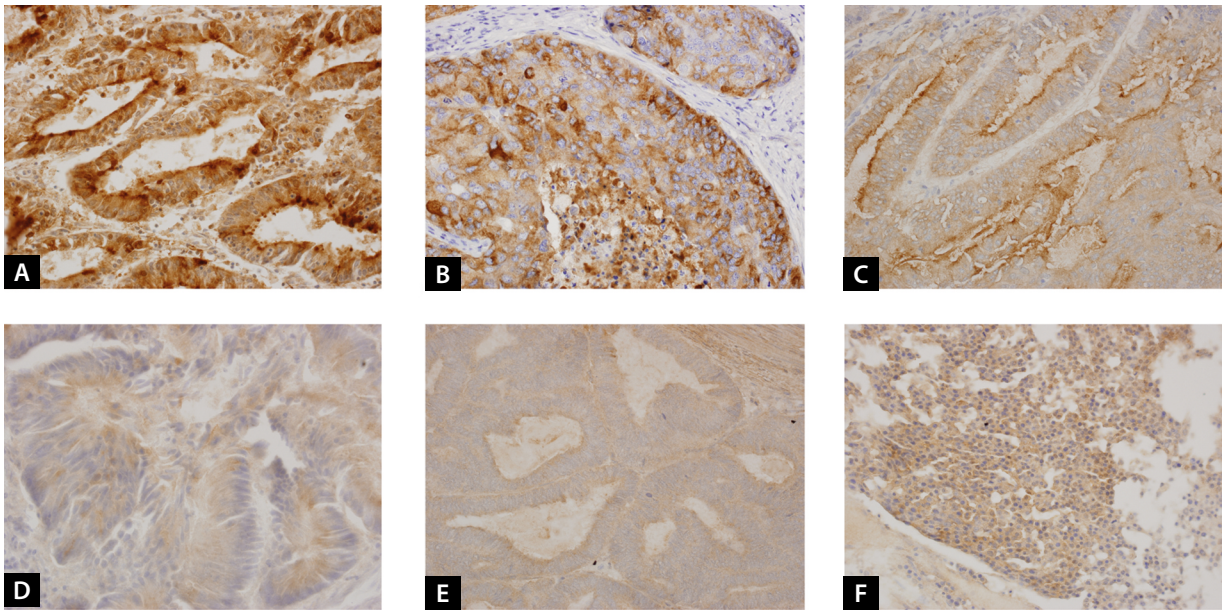

Figure 1. Representative microphotographs of B7–H4 immunoreactivity in endometrial cancer; **A.** Endometrioid endometrial carcinoma with strong apical and cytoplasmatic B7–H4 expression; **B.** Endometrioid endometrial carcinoma with strong cytoplasmatic membranous B7–H4 expression; **C.** Endometrioid endometrial carcinoma with weak cytoplasmatic and membranous B7–H4 expression; **D.** Endometrioid endometrial carcinoma with weak apical B7–H4 expression; **E.** Endometrioid endometrial carcinoma with weak apical B7–H4 expression; **F.** Serous endometrial carcinoma with moderate cytoplasmatic and membranous B7–H4 expression

labelled cells; 2 = 31–60% of labelled cells; 3 ≥ 60% of labelled cells. The final IRS score was calculated as a multiplication of the staining intensity and the percentage of labelled cells (A × B). The final score ranged from 0 to 6.

Statistical analysis

Non-parametric tests (Mann-Whitney or Kruskal-Wallis) were used for evaluation of median B7–H4 IRS between analysed groups. The cut off value to discriminate between low and high B7–H4 immunoreactivity was set at median IRS. High IRS was considered as equal to greater than the median IRS, while low IRS was considered as less than the median. Survival analyses were conducted using the Kaplan-Meier survival curves and the differences in patient survival were compared using the log-rank test. Multivariate survival analysis was conducted using Cox proportional-hazards regression with the backward method of variable entry. We used the following confounders for model development: lymph node metastases, tumor grade, tumor stage, high B7–H4 IRS. Statistical analysis was conducted using MedCalc 11.4.2.0., MedCalc Software, Seoul, Republic of Korea, and GraphPad InStat 3.06, GraphPad Software Inc., San Diego, CA, USA.

RESULTS

Patients' characteristics

We identified 63 patients who met the inclusion criteria. The median patient age was 67 and the range from 47 to 92 years. The study group included 47 patients with low grade endometrioid endometrial carcinomas, 10 with high grade endometrioid endometrial cancers, 3 with serous, and 3 with clear cell endometrial carcinomas. The TNM classification of the T of the tumors was as follows: T1a — 16 patients; T1b — 29 patients; T2 — 12 patients; T3a — 1patients; T3b — 4 patients; and T4 — 1 patient. The median follow-up period for patients was 5 years. The median number of harvested lymph nodes was 15 (interquartile range, IQR = 8 – 20). Lymph node metastases were found in 9 (14%) patients.

B7–H4 immunoreactivity

B7–H4 reactivity was observed in all, except one, endometrial cancer patients (98%). Positive apical membranous expression was observed in 14 cases (22%), cytoplasmatic and membranous expression in 37 cases (59%) and both in 11 cases (17%). Staining intensity was as follows: no reaction in 1 case, weak in 16 (24%) patients, moderate in 25 (37%) patients, and strong in 22 (35%) patients. Twenty-nine (46%) patients presented B7–H4 immunoreactivity in more than 60% of cells, while, in the case of 18 (29%) and 16 (25%) patients, the percentage of labelled cells were 30–60% and < 30%, respectively. Median IRS was 2 (range 0–6). High IRS for B7 H4 was found in 41 patients (65%), while low B7–H4 IRS was observed in 22 (35%) patients. We did not find any relationship between B7–H4 IRS and the histopathological type of the tumor, lymph node

Table 1. Relationship between the B7–H4 immunoreactivity score (IRS) and clinicopathological characteristics of endometrial cancer patients

	Median B7–H4 IRS	Range	p value
Histopathological type			
Endometrioid low-grade (n = 47)	2.0	0–6	0.28
Endometrioid high-grade (n = 10)	3.5	0–6	
Non-endometrioid (n = 6)	0	0–6	
Lymph node metastases			
No (n = 54)	3.0	0–6	0.45
Yes (n = 9)	2.0	0–6	
Myometrial invasion			
Below 50% (T1a; n = 16)	3.0	0–6	0.31
Above 50% (T1b; n = 29)	3.0	0–6	
FIGO stage of the disease			
I (n = 45)	3.0	0–6	0.59
II (n = 12)	2.0	0–6	
III and IV (n = 6)	3.0	0–6	

FIGO — International Federation of Gynecology and Obstetrics

metastases, depth of myometrial invasion, or International Federation of Gynecology and Obstetrics (FIGO) stage of the disease. Detailed results are summarized in Table 1.

Survival analyses

We found significantly worse OS of patients with high versus low B7–H4 IRS (p = 0.03). In the patient group with high B7–H4 IRS, the median OS was 60 months (range 2–92 months), while in the group with low B7–H4 IRS the median OS was not reached (range 34–91 months). Five-year OS was 69% and 92% in the group of patients with high and low B7–H4 IRS respectively. Survival curves are presented in Figure 2A. In the multivariate, adjusted, survival analysis, we found that the presence of lymph node metastases [Hazard Ratio (HR) = 2.9; 95% confidence interval (CI) 1.03–8.16, p = 0.045], tumor stage (HR = 3.14; 95% CI 1.79–5.49; p < 0.01) were independently related with shortened overall survival. The association between high B7–H4 IRS and shortened patient survival approached the level of significance (HR = 4.43; 95% CI 0.99–19.76; p = 0.052). In our cohort, tumor grade was not associated with patient survival. We found no difference in patient's survival in relation to the type of B7–H4 immunoreactivity (p = 0.87, Fig. 2B). Across all three groupings, the median OS was not reached. The range of survival was as follows: apical membranous expression 3–89 months, cytoplasmatic and membranous expression 2–88 months, and in cases with both type of expression 37–92 months. Five-year OS were 86%, 74% and 91% respectively.

DISCUSSION

B7–H4 is excessively produced in many tumors, including breast cancer, cervical cancer, and some types of ovarian cancers [15–17]. However, data regarding B7–H4 expression in endometrial cancer are sparse and those studies that have been reported are consistent with each other. Miyatake et al. [18], reported B7–H4 immunoreactivity in all of the specimens of normal and hyperplastic endometrium as well as in malignant cells, nevertheless the intensity and percentage of B7–H4 staining in hyperplastic to malignant endometrial cells was higher than for a normal endometrium. Also, in the study of Qian et al. [19], the intensity of reaction in normal cells was less intense when compared to cancer cells. These findings are not consistent with those obtained by Liu et al. [20], who also reported B7–H4 immunoreactivity in all of the normal and malignant specimens, however, there were no differences in the expression levels of B7–H4 between endometrial cancer and normal endometrium, with both classified as high. Similarly, Vanderstraeten et al. [21], detected B7–H4 in all cases of normal endometrium and primary endometrial carcinoma, as well as in recurrent endometrial carcinoma, and in 90% of metastatic endometrial carcinoma. These results were consistent with our study, while we found that B7–H4 was

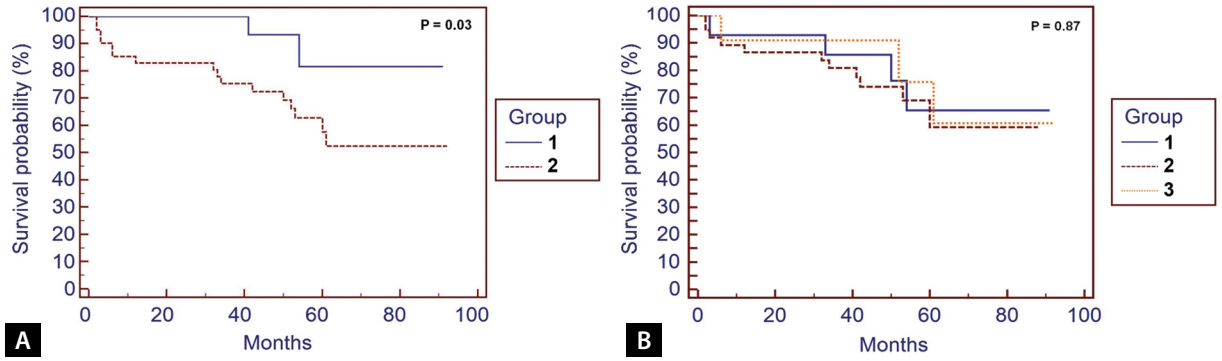

Figure 2. Survival curves of endometrial cancer patients according to B7H4 immunoreactivity; **A.** Survival curves of patients with low and high B7–H4 immunoreactivity score (IRS). Group 1. Low B7–H4 IRS, median overall survival (OS) was not reach, 5-year OS 92%. Group 2. High B7–H4 IRS, median OS — 60 months, 5-year OS 69%, p = 0.03; **B.** Survival curves according to the type of B7H4 immunoreactivity. Group 1. apical membranous expression, mOS — not reached, range: 3–89 months, 5-year OS was 86%. Group 2: cytoplasmatic and membranous expression, mOS — not reached, range: 2–88 months, 5-year OS was 74%. Group 3: both type of expression, mOS — not reached, range: 37–92 months, 5-year OS was 91%. p = 0.87

expressed in 98% of endometrial cancers and moderate or strong expression was observed in most patients. Similarly, Bregar et al. [22], observed a prevalence of B7–H4 in endometrial cancer cell. The incidence of B7–H4 expression in our study and in those previously mentioned was higher than that reported in the study by Zong et al. [23], where B7–H4 immunoreactivity only occurred in 71.5% of endometrial cancers.

B7–H4 is a transmembrane protein, but its presence in the intracellular compartment has also been reported in some tumors [24, 25]. Membrane B7–H4 may be responsible for tumor progression as an effect of suppressing T-cell immunity, while intracellular B7–H4 probably does not have such a function though its precise role is not well known [24]. However, it was shown that intracellular B7–H4 promotes cell proliferation, adhesion and invasion [26]. It may also suppress apoptosis [8]. Membranous B7–H4 has an impact on decreased density of tumor infiltrating lymphocytes (TILs), whereas intracellular B7–H4 has no such effect [27]. A high number of TILs seems to be related with improved prognosis in cancer patients [10]. In our study we observed cytoplasmic and membranous reaction in the main. Similarly in the other studies, B7–H4 expression in endometrial cancer is described as cytoplasmic and circumferential membranous, whereas a predominantly apical reaction was mostly detected in normal endometrial tissue [18–20]. In our study, we found no difference in either patient prognosis or clinicopathological features of the tumors regarding different locations of B7–H4 immunoreactivity. Therefore, the biological significance of different sites of B7–H4 immunoreactivity requires further research.

Previous studies including patients suffering from colorectal, gastric, lung, prostate, and thyroid cancers, and melanoma and osteosarcoma reported that high expression of B7–H4 was correlated with the tumor's depth of invasion, distant metastasis, cancer progression, higher recurrence rate, or poorer patient outcome [14, 25, 28–30]. In the present study we analysed the expression of B7–H4 in endometrial cancer cells and its impact on patient survival, histopathological type of the tumor, lymph node metastases, depth of myometrial invasion, and FIGO stage of the disease. We observed a significantly worse prognosis of patients with high B7–H4 immunoreactivity when compared to patients with low B7–H4 immunoreactivity. However, in multivariate analysis, when other risk factors were included, the significance of B7–H4 was only borderline (p = 0.052). In contrast to our result, Miyake et al. [18], did not observe any impact from the percentage of positive cells and the intensity of staining on disease free survival [18]. Furthermore, in the study by Zong et al. [23], the authors even found improved patient survival in cases of endometrial cancer with B7–H4 expression. That association was significant both in the whole study group, and in the NSMP subtype. However, they observed a more favourable histopathological profile (low grade, stage I of the disease, and endometrioid histology) in B7–H4 expressing endometrial cancers [23]. On the contrary, in our study, we found no relationship between B7–H4 IRS and the histopathological type of the tumor, lymph node metastases, depth of myometrial invasion, or FIGO stage of the disease. Additionally, the grade of the tumor (high vs low) was not associated with B7–H4 expression. Similar results were also obtained by Bregar et al. [22], whose authors found no relationship between B7–H4 expression and tumor grade and histopathological type. Furthermore, they reported that B7–H4 expression was independent of MSI status [22].

Tumors classified as MSI are usually insensitive to chemotherapy, however they produce many antigens because of a huge number of mutations. High tumor mutation burden result in the expression of numerous tumor antigens, and therefore, these tumors are more prone to immunotherapy [31]. PD-1, a receptor for PD-L1 (B7–H1) was shown to be a good target for immunotherapy in endometrial cancer [32]. Recently the Food and Drug Administration (FDA) approved pembrolizumab and dostarlimab, both anti-PD-1 antibodies, for therapy of advanced endometrial cancer that is MSI-H [33, 34]. Considering high B7–H4 expression in endometrial cancers, this immune checkpoint inhibitor also seems to be a promising target for immunotherapy in endometrial cancer, and some clinical trials are currently in progress [8, 32].

In summary, there are many conflicting data on B7–H4 expression in endometrial cancer. It may be due to the retrospective character of available studies and the subjectivity of immunohistochemical evaluation. In addition, in all the research mentioned has differed regarding antibodies against B7–H4, scoring systems, and cut off values, all of which might have influenced the results of the studies. The same problems are reported in the evaluation of PD-L1 expression as a predictive biomarker for sensitivity to immune checkpoint blockade.

Similarly, the main limitation of our study is its retrospective character and usage of subjective immunoreactivity assessment. Additionally, as we mentioned above, there is no any universal B7–H4 immunoreactivity scoring system available in the literature, so we just decided to use one of previously used. Therefore, this problem requires further research [35].

CONCLUSIONS

Our result suggests that B7–H4 expression might be a useful prognostic factor in endometrial cancer.

Article information and declarations

Data availability statement

Available on request.

Ethics statement

The study was approved by Ethical Committee of Centre of Postgraduate Medical Education, Warsaw (84/PB/2020).

Author contributions

Katarzyna Gorzelnik — 50% contribution, concept and article writing.

Anna Wasążnik-Jędras — 15% contribution, article writing, IHC analysis.

Łukasz Wicherek — 15% contribution, concept and article revision.

Sebastian Szubert — 20% — statistical analysis, writing.

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Conflict of interest

The authors assert they have no conflicts of interest to declare.

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Metformin-associated maternal and neonatal outcomes in women with gestational diabetes — a retrospective cohort study

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ABSTRACT

Objectives: To assess the maternal and neonatal outcomes in women with GDM treated with metformin, medical nutrition therapy (MNT) or insulin.

Material and methods: The current retrospective cohort study includes data from 233 women diagnosed with GDM who gave birth between January 2017 and January 2019 at an obstetrics and gynecology hospital in Sofia, Bulgaria. Patients were assigned to three groups, according to the treatment approach — metformin group (n = 70), insulin group (n = 40), and MNT group (n = 123). Values of fasting plasma glucose (FPG) and glycated hemoglobin (HbA1c) have been evaluated at diagnosis of GDM and the third trimester of pregnancy. A comparative analysis of pregnancy outcomes and short-term neonatal characteristics in the investigated groups has been performed.

Results: Women indicated for pharmacological treatment (metformin or insulin) had significantly higher BMI ($p < 0.01$), FPG ($p < 0.001$), and HbA1c levels ($p < 0.001$) at baseline. However, during pregnancy, patients treated with metformin showed a significantly lower BMI ($p < 0.01$), FPG ($p < 0.01$), and HbA1c ($p < 0.01$). Neonates born to metformin-treated mothers had lower birth weight compared to those born to women in the MNT and insulin groups (metformin vs MNT, $p < 0.001$; metformin vs insulin, $p = 0.03$). The lowest incidence of newborns with macrosomia and neonatal hypoglycemia has been observed in the metformin cohort. Not a single newborn with an Apgar score under 7 has been identified in the metformin group.

Conclusions: According to the current analysis, women with GDM treated with metformin demonstrated better maternal and neonatal outcomes. No short-term complications in newborns have been associated with metformin use during pregnancy.

Keywords: gestational diabetes; metformin; insulin; maternal outcomes; neonatal outcomes

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INTRODUCTION

Gestational diabetes mellitus (GDM) is the most common cause of hyperglycemia during pregnancy [1]. According to the International Diabetes Federation (IDF), one in six live births (16.7%) in 2021 were to women with hyperglycemia during pregnancy, most often (80.3%) due to GDM [2]. The prevalence of GDM was estimated to vary between 1 and 28% according to different population studies [3]. More than 50% of women with previous GDM are at higher risk of developing type 2 diabetes mellitus (T2DM) in the first five years after birth [4].

Hyperglycemia in pregnancy is a risk factor for maternal and fetal complications. Pregnancies complicated with GDM are linked with an elevated risk of hypertensive disorders and cesarean deliveries [1]. In addition, women with GDM have a 10-fold higher risk of developing T2DM later in life

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compared to normoglycemic pregnancies. [5]. Women with a history of GDM are 30–84% more likely to develop it again in a subsequent pregnancy [6]. The most common perinatal and neonatal complications associated with GDM include macrosomia, shoulder dystocia, respiratory distress syndrome, neonatal hypoglycemia, polycythemia, and hyperbilirubinemia [1]. Exposure to maternal hyperglycemia increases the risk of childhood overweight and obesity associated with the development of T2DM [7].

Treatment of GDM has been proven to reduce the risk of perinatal complications by 75% and fetal macrosomia by 50% [8]. Lifestyle modification (diet and physical activity) is essential to GDM management. According to the American Diabetes Association (ADA) guideline, 70–85% of women with GDM, can maintain adequate glycemic control with lifestyle modification alone [9]. If blood glucose targets have not been achieved by changes in diet and exercise within 1–2 weeks, pharmacotherapy should be further initiated [9, 10]. Up to 30% of patients with GDM may require pharmacological therapy [11].

Since insulin does not cross the placenta, it is recognized by many guidelines as a first-line pharmacological option for GDM treatment [9, 12, 13]. According to the International Federation of Gynecology and Obstetrics (FIGO) Initiative on GDM, insulin is considered the first-line treatment in women with dysglycemia, especially those at high risk of failure of oral antidiabetic therapy. In addition, other factors associated with the need for insulin therapy noted in FIGO guideline include hyperglycemia detected before 20 weeks of gestation; fasting plasma glucose (FPG) > 6.1 mmol/L or post-prandial glucose levels > 7.8 mmol/L; and increased pregnancy weight gain (> 12 kg) [1]. After 30 weeks of gestation, pharmacological therapy has often been needed [1, 9, 14]. Despite the predictive factors mentioned above, there is no universal consensus regarding the timing of the initiation of pharmacotherapy for pregnant women with GDM [15].

Although insulin is an effective and safe treatment approach during pregnancy, it is associated with the risk of several adverse outcomes: hypoglycemic episodes, weight gain, and the requirement for multiple daily glucose self-monitoring. In addition, insulin treatment requires special storage conditions and patient education regarding proper injection technique. Patient compliance and adherence are crucial determinants of insulin therapy effectiveness [16].

In comparison to insulin, oral hypoglycemic agents have several advantages like low cost, easier administration, and better patient compliance [16]. The two oral antidiabetic medications reported to be used to treat GDM are metformin and glyburide [17].

Metformin is a biguanide that is the most widely prescribed hypoglycemic medication, currently included in the World Health Organization's Model List of Essential Medicines. It is the first-line monotherapy for the treatment of T2DM [18]. Metformin does not enhance insulin secretion which is associated with a lower risk of hypoglycemia [19]. Several studies have demonstrated the benefits of metformin use for the prevention and treatment of GDM. However, metformin use during pregnancy may rise concerns due may be controversial due to its ability to cross the placenta [20].

According to several practice guidelines, such as FIGO, the UK National Institute for Health and Care Excellence (NICE), the Endocrine Society, and the German Diabetes Association, metformin can be considered as the first-line option for the pharmacological treatment of GDM. [1, 10, 13, 21]. In contrast, other professional organizations like ADA, and the American Congress of Obstetricians and Gynecologists (ACOG), do not recommend metformin as first-line treatment, because of its transplacental transport and lack of data on long-term safety [9, 14]. Although these concerns, ACOG guidelines consider that in some cases, metformin could be a reasonable alternative to insulin [14].

Objective

This study aims to comparatively assess the maternal and neonatal outcomes in women with GDM treated with metformin, medical nutrition therapy (MNT), or insulin.

MATERIAL AND METHODS

Study design and setting

The current retrospective observational cohort study is based on the electronic records of the validated integrated information system of a specialized obstetrics and gynecology hospital with national coverage of patients in Bulgaria (Joystick, ver. 2.1). The study has been considered by the Institutional Review Board of Specialized Obstetrics and Gynecology Hospital "Dr Shterev", Sofia Bulgaria. The research project has been conducted in accordance with ethics and law standards for medical research, as stated in active national legislation and the Declaration of Helsinki. The proposed non-interventional retrospective database does not jeopardize the confidentiality and autonomy of any patients.

Study population

Electronic medical records of 233 pregnant women diagnosed with GDM between January 2017 and January 2019 have been analyzed. GDM was diagnosed with a 2-h 75-g oral glucose tolerance test (OGTT) using the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) [22] and American Diabetes Association (ADA) [9] criteria. Early GDM screening (before 20 weeks of gestation) was performed in 109 patients due to a family

history of diabetes mellitus and high body mass index (BMI). The remaining 239 women underwent universal screening at 24–28 weeks of gestation.

Pregnant women aged between 18–40 years old, diagnosed with GDM, and with a singleton pregnancy were included in the analysis. Women with multiple pregnancies and those with pre-existing diabetes (types 1 and 2) have been excluded from the study.

Patients were divided into three groups, depending on the treatment approach – metformin group (n = 70), insulin group (n = 40), and MNT group (n = 123). Only women who did not change the therapeutic strategy until the end of pregnancy were included in the study.

In 33 patients, metformin was started before pregnancy, due to evidence of insulin resistance (IR), most often in the background of polycystic ovary syndrome (PCOS). The metformin treatment was discontinued a week before the OGTT was performed and started again in those patients who met the criteria for GDM diagnosis. In the remaining 37 women included in the metformin group, the oral hypoglycemic therapy was started up to two weeks after GDM diagnosis if the glycemic target was not met with lifestyle modification (diet and exercise changes) alone. The decision to initiate metformin was also based on the pregnant woman's weight gain, medication tolerability and willingness to undergo oral hypoglycemic therapy. Target levels for blood glucose measurements were adopted from ADA guideline and were as follows: FPG < 5.3 mmol/L; 1-h postprandial glucose < 7.8 mmol/L or 2-h postprandial glucose < 6.7 mmol/L [9]. All women receiving metformin were aware by the endocrinologist of the benefits and risks of the off-label treatment and gave their informed oral consent. The initial dose of metformin varied from 500 mg to 1500 mg daily. The dose was subsequently titrated up to a maximum of 1500 mg daily to achieve target blood glucose levels. Insulin treatment was initiated when blood glucose targets were not met with metformin. But as noted above those patients who switched the therapeutic approach were excluded from the study.

Insulin was selected as first-line therapy in pregnant women who refused metformin therapy and in those who did not meet the glycemic target for pregnancy through lifestyle modification alone. In most cases (n = 32), only basal insulin (detemir) was administered to maintain normal FPG levels. The remaining patients (n = 8) were treated with a basal-bolus regimen receiving insulin aspart at meals and insulin detemir once daily.

Measurements and laboratory data

A comparative analysis of the maternal characteristics, pregnancy outcomes and neonatal characteristics of the three groups, has been performed. The following data, extracted from medical records, were analyzed:

- **maternal characteristics and pregnancy outcomes** — age, BMI, values of FPG, HbA1c, family history of diabetes, previous history of GDM, parity, conception mode, gestation age at delivery, mode of delivery, the incidence of pregnancy-induced hypertension or preeclampsia, the incidence of PCOS;
- **neonatal outcomes** — birth weight, macrosomia, baseline APGAR scores after delivery, neonatal hypoglycemia, shoulder dystocia, small-for-gestational-age (SGA), and respiratory distress.

The maternal characteristics, including BMI, values of fasting plasma glucose and HbA1c, were measured at the time of GDM diagnosis and the end of pregnancy. All observed women were Caucasian.

Statistical analysis

Data analysis was performed using the Statistical Package for Social Sciences (SPSS) v.20.0. Continuous variables are expressed as the mean ± standard deviation, and categorical variables are presented as numbers and percentages. Continuous variables were compared between the groups using Mann–Whitney U-test. Fisher's exact test and Pearson's chi-square test were used for categorical variables. The p values less than 0.05 were considered statistically significant.

RESULTS

The maternal characteristics of the observed women are presented in Table 1. Patients who needed pharmacological therapy showed significantly higher BMI at baseline. The mean FPG levels were significantly lower in the MNT group (5.27 ± 0.67 mmol/L) and metformin group (5.72 ± 0.80) compared to the insulin group (6.69 ± 0.74 mmol/L) (Tab. 1, Fig. 1A). Patients who needed pharmacological therapy showed significantly higher mean FPG levels ($p < 0.01$). Similar findings have been identified when HbA1c levels at baseline were compared (Tab. 1, Fig. 1B).

Spontaneous pregnancies predominated in metformin and MNT groups (51.4% vs 53.7%). Significant differences have been identified regarding GDM incidence in primiparous pregnancies. Regarding the mode of delivery, caesarean sections predominated in all three groups. No statistically significant differences have been found in gestational weeks at birth.

Neonatal birth weight in the metformin-treated group was lower compared to the insulin group (3154.13 ± 463 g vs 3421.79 ± 553 g, $p = 0.03$) and MNT group (3154.13 ± 463 g vs 3323.66 ± 521 g, $p < 0.01$). The biparietal diameter was larger in newborns in both the insulin and MNT groups. How-

Table 1. Maternal characteristics and biochemical measures

Maternal characteristics	Metformin group (n = 70)	Insulin group (n = 40)	MNT group (n = 123)	p value metformin vs insulin	p value metformin vs MNT
Age [years]	36.8 ± 4.9	34 ± 3.8	35.13 ± 4.3	0.01*	0.02
BMI [kg/cm^2] (1st trimester)	27.83 ± 5.351	27.17 ± 5.21	25.70 ± 5.81	NS	< 0.01*
BMI [kg/cm^2] (3rd trimester)	28.96 ± 4.21	30.53 ± 4.202	28.65 ± 3.98	NS	NS
Parity, n (%) Primiparous Multiparous	 38 (54.3%) 32 (45.7%)	 27 (67.5%) 13 (32.5%)	 68 (55.3%) 55 (44.7%)	0.02*	NS
Mode of conception, n (%) Spontaneous ART	 36 (51.4%) 34 (48.6%)	 18 (45.0%) 22 (55.0%)	 66 (53.7%) 57 (46.3%)	NS	NS
Family history of diabetes, n (%) Yes No	 42 (60.0%) 28 (40.0%)	 27 (67.5%) 13 (32.5%)	 55 (44.7%) 68 (55.3%)	NS	0.04*
FPG [mmol/L] (baseline)	5.72 ± 0.801	6.69 ± 0.74	5.27 ± 0.679	< 0.01*	< 0.01*
FPG [mmol/L] (3rd trimester)	5.06 ± 0.784	5.41 ± 0.723	5.3 ± 0.751	< 0.01*	< 0.01*
HbA1c [%] (baseline)	5.61 ± 0.9	6.29 ± 0.35	5.34 ± 0.32	< 0.01*	< 0.01*
HbA1c [%] (3rd trimester)	5.27 ± 0.9	5.58 ± 0.42	5.39 ± 0.31	< 0.01*	< 0.01*

*Statistically significant difference; ART — Assisted Reproductive Technology; BMI — body mass index; FPG — fasting plasma glucose; GDM — gestational diabetes mellitus; MNT — medical nutrition therapy; NS — not statistically significant difference; Data are presented as number (percentages) or mean ± SD

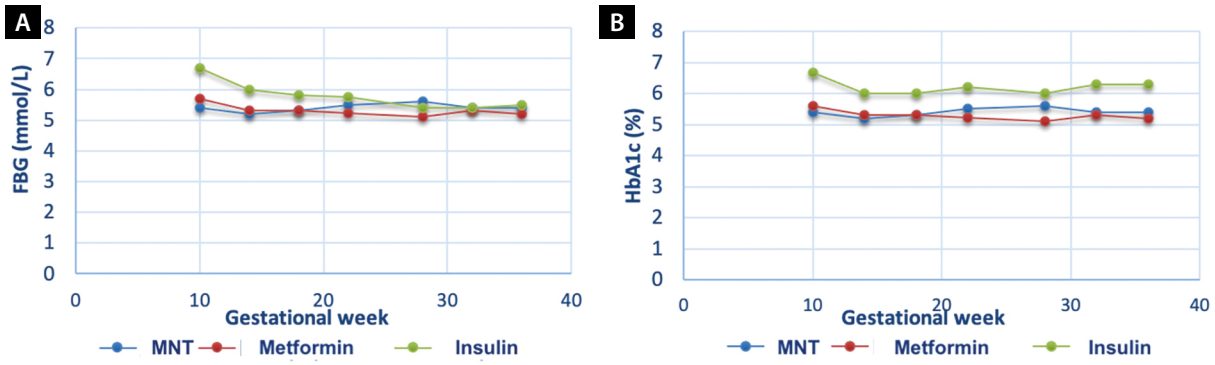

Figure 1. Glycemic control among the three groups of observed women; **A.** Fasting plasma glucose (FPG) [mmol/L] at diagnosis of gestational diabetes mellitus (GDM) and during the course of pregnancy; **B.** HbA1c [%] at diagnosis of GDM and during the course of pregnancy; MNT — medical nutrition therapy

ever, a statistically significant difference was found only in the metformin vs MNT group (p < 0.01). A significant difference in the newborn length has been identified comparing the metformin and insulin groups (p = 0.04) (Tab. 2). The lowest incidence of macrosomia (2.9%) and neonatal hypoglycemia (2.9%) was observed in the metformin group. A statistically significant difference was found regarding macrosomia incidence in the metformin vs insulin group (p = 0.01). The incidence of small-for-gestational-age (SGA) neonates in the three groups was similar. There were no newborns with baseline Apgar score under 7 in the metformin-treated group (Tab. 2).

DISCUSSION

In recent years, the benefits and risks of metformin use in pregnant women have been widely discussed. The main concern associated with metformin therapy in GDM is caused by the transplacental transport. Metformin has

Table 2. Pregnancy and neonatal outcomes

	Metformin group (n = 70)	Insulin group (n = 40)	MNT group (n = 123)	p value metformin vs insulin	p value metformin vs MNT
Pregnancy outcome					
Delivery mode, n (%) Vaginal Cesarian section	 21 (30.0%) 50 (70.0%)	 8 (20.0%) 32 (80.0%)	 36 (29.3%) 87 (70.7%)	NS	NS
Gestational age at delievery [weeks]	38.2 ± 1.2	37.6 ± 0.8	38.5 ± 1.5	NS	NS
Neonatal outcome					
Birthweight [g]	3154.13 ± 463	3421.79 ± 553	3323.66 ± 521	0.03*	< 0.01*
Length [cm]	49.40 ± 3.1	50.18 ± 2.13	49.69 ± 3.9	0.04*	NS
Biparietal Diameter [cm]	92.22 ± 2.1	93.76 ± 3.1	95.11 ± 3.2	NS	< 0.01*
Macrosomia	2 (2.9%)	6 (15.0%)	11 (8.9%)	0.01*	NS
SGA	6 (8.5%)	4 (10%)	4 (4.9%)	NS	NS
Neonatal hypoglycemia	2 (2.9%)	3 (7.5%)	6 (4.9%)	NS	NS
Baseline Apgar score < 7	0 (0%)	2 (5%)	0 (0%)	0.05*	–

*Statistically significant difference; MNT — medical nutrition therapy; NS — not statistically significant difference; SGA — small for gestational age; Data are presented as number (percentages) or mean ± SD

been shown to cross the placenta and its concentration in the umbilical cord at the time of delivery can reach more than 50% of the maternal concentrations [23].

There are observed differences in metformin effects during each trimester of pregnancy. During the first trimester, the embryo has much fewer, albeit more active mitochondria. Therefore, many clinicians prefer to use metformin until the end of the first trimester. In the later stages of pregnancy, the use of metformin may be associated with reduced nutrient supply to the fetus. This could be a prerequisite for delivering a newborn with a lower weight, which corresponds with the results listed above. However, meta-analyses show that metformin improves maternal glycemic control and insulin sensitivity, reduces pregnancy weight gain and fetal insulin resistance [24]. According to the available data, metformin is considered a non-teratogenic drug [25]. A meta-analysis conducted by Gilbert et al. [26] shows that there is no evidence of an increased risk for major malformations when metformin is taken during the first trimester of pregnancy.

As noted, in our study, in 33 patients, metformin treatment was a continuation of therapy started before pregnancy due to evidence of IR, most commonly in the background of PCOS. All pregnant women underwent aneuploidy screening by the end of the first trimester and fetal morphology scanning between 19–23 and 30–32 weeks of gestation. No teratogenic effect of metformin use in these pregnant women has been observed. In the remaining women (n = 37), oral therapy was initiated after the diagnosis of GDM. In all of them, metformin was started after the end of the first trimester. No side effects or complications for both the mother and the fetus were found. In our analysis women treated with metformin showed a significant improvement in glycemic control and less weight gain during pregnancy.

Over the past two decades, several studies have discussed the short- and long-term effects of metformin use in GDM. Metformin use during pregnancy was first studied in a cohort study involving 118 pregnant women with type 2 diabetes and GDM [27]. Due to increased perinatal mortality with metformin in the third trimester compared with insulin (11.6% vs 1.3%, $p < 0.02$), many clinicians are suspicious to consider metformin as an alternative to insulin until the results of the first large, randomized trial (Metformin in Gestational Diabetes - MiG) were published in 2008 [28]. The study was conducted in Australia and compared pregnancy outcomes in 751 women with GDM treated with metformin and insulin and divided into two groups. The results regarding neonatal hypoglycemia, respiratory distress syndrome, birth trauma, and premature birth were similar in both groups. In the metformin-treated

group was established less weight gain during pregnancy. No serious adverse outcomes associated with metformin have been observed [28].

The current study confirms the acceptable efficacy and safety profile of metformin for both the mother and the newborn. No short-term complications in the group treated with metformin were observed. During pregnancy, patients treated with metformin showed lower BMI, lower FPG, and lower levels of HbA1c ($p < 0.01$) compared to the insulin group. Our findings support observations from a previous study, conducted by McGrath et al. [29]. The results from this retrospective, case-control study show that women managed with metformin had a higher early pregnancy BMI compared to those receiving insulin or diet and lifestyle modification ($p < 0.001$). Pregnant women, successfully managed by diet and lifestyle modification had significantly lower FPG levels ($p < 0.001$) and HbA1c ($p < 0.01$) at diagnosis of GDM. Similar findings are generated by our analysis. Furthermore, the authors have observed that there were no differences regarding mode of delivery, birth weight or incidence of large/small-for-gestational-age neonates between the three groups [29].

A similar research design comparing three groups of women with GDM divided regarding the used treatment approach was adopted by several other authors [30, 31]. Tertti et al. [30] suggested that metformin is an effective treatment option for women with GDM and does not seem to be associated with higher risks for maternal or neonatal complications compared with insulin. In an observational study from New Zealand, Goh et al. [31] concluded that the use of metformin in the treatment of GDM was associated with fewer adverse pregnancy outcomes compared with insulin.

CONCLUSIONS

Current observations confirm that metformin improves maternal and neonatal outcomes in women with GDM and mild hyperglycemia. Nevertheless, metformin cannot replace insulin treatment in every GDM patient. The results from this retrospective study revealed that women with higher baseline BMI needed further pharmacological therapy to maintain euglycemia. Women with GDM, treated with metformin had a more favorable profile for all the investigated criteria. Exposure to metformin is not associated with short-term adverse maternal and neonatal outcomes.

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Conflict of interest

The authors declare no conflict of interest.

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Inflammation in recurrent miscarriage — a comprehensive perspective from uterine microenvironment and immune cell imbalance to therapeutic strategies

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ABSTRACT

Recurrent miscarriage, poses a significant challenge for many couples globally, the causes of which are not fully understood. Recent studies have shown the intricate link between uterine inflammation and recurrent miscarriages. While inflammation is essential during early pregnancy stages, especially in embryo implantation, an imbalance can lead to miscarriage. Key inflammatory mediators and an imbalance in immune cells can significantly alter and contribute to recurrent miscarriages. Lifestyle factors like smoking and obesity exacerbate inflammatory responses, increasing miscarriage risks. Understanding the interaction between the uterine environment, immune cell imbalances, and recurrent miscarriages is essential for devising effective treatments. This paper presents the latest data on inflammation's role in recurrent miscarriage, emphasizing the significance of diagnosing chronic endometritis and immune imbalances, offering practical recommendations for treatment and diagnosis.

Keywords: endometrium; immune cells; recurrent miscarriage; inflammatory cytokines

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INTRODUCTION

Recurrent miscarriage, which refers to experiencing two or more consecutive pregnancy losses, is a big challenge for many couples around the world [1]. In recent years, there has been a lot of attention on the complex relationship between inflammation in the uterus and recurrent miscarriages [2]. Under typical physiological conditions, inflammation plays a pivotal role during the early stages of pregnancy, especially in embryo implantation [3]. However, when this inflammatory balance is disrupted, particularly within the uterine lining, key inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and interleukin-6 (IL-6) can change significantly in concentration and potentially lead to recurrent miscarriages [4, 5]. Moreover, lifestyle and environmental factors such as smoking and obesity have been proven to increase inflammatory responses and raise the risk of miscarriage [6]. The imbalance of immune cells during this process might also be a critical component [7, 8]. Therefore, it is extremely important to fully understand how the uterine microenvironment interacts with immune cell imbalances and recurrent miscarriages in order to develop targeted therapeutic strategies that can provide more effective treatments for affected couples.

This article explores the role of inflammation in recurrent miscarriage, with a focus on the uterine microenvironment and immune cell imbalance. Chronic endometritis (CE) can contribute to this imbalance, and antibiotic therapy is considered the treatment of choice for CE. The article emphasized the importance of accurate diagnosis of CE and immune imbalance and provides clinicians with the treatment and diagnosis of practical recommendations.

ENDOMETRIUM MICROENVIRONMENT

The endometrium underwent cyclical changes in preparation for pregnancy, creating a microenvironment that was favorable for embryo implantation. A balanced inflammatory response was essential for successful implantation. However, prolonged or excessive inflammation could hinder

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normal embryo implantation and development. Chronic endometritis, characterized by sustained inflammation, had significant implications for a woman's reproductive outcomes. Research indicated that chronic endometritis exacerbated uterine fibrosis and negatively impacted reproductive outcomes, especially in women with moderate to severe intrauterine adhesions and those who experienced recurrent early pregnancy loss [9]. Additionally, chronic endometritis was frequently implicated in repeated implantation failures, suggesting its pivotal role in miscarriages [7, 10–15]. Additionally, chronic endometritis was frequently implicated in repeated implantation failures, suggesting its pivotal role in miscarriages [16]. The endometrial microbiome was crucial for the success or failure of embryo implantation. Investigations revealed that these microorganisms were not solely of vaginal origin but maintained a distinct presence in the endometrium. Hormonal modulation might have influenced these microbial populations within endometrial epithelial cells. In the realm of assisted reproductive technology, alterations in the microbial composition at the embryo-endometrium interface could influence the success of embryo implantation [17]. In particular, the disorder of endometrial microbial community is lead to the potential cause of the failure of the blastocyst implantation in patients with in vitro fertilization [18]. Moreover, dysregulation of the endometrial microbiome has been linked to other pregnancy pathologies, underscoring the importance of microbiome balance for a successful pregnancy [19, 20]. Notably, after antibiotic treatment, the pregnancy outcomes of these women with chronic endometritis showed significant improvement [12, 21, 22]. These findings underscore a definitive relationship between chronic endometritis and miscarriage, and appropriate treatment might enhance the reproductive outcomes for women.

The relationship between endometritis and miscarriage has been confirmed in multiple studies. Inflammation plays a crucial role in normal implantation and pregnancy processes [15]. However, when the inflammatory response is excessive, it may lead to miscarriage. This excessive inflammation might be due to a deficiency in the number of CD56brightCD16– natural killer (NK) cells in the endometrium or their impaired function [23]. These NK cells are the primary regulators of inflammation levels at the placental interface [24]. They can be stimulated by placental fragments and apoptotic cells, leading to the production of inflammatory cytokines. When there is an excessive number of placental fragments and apoptotic cells, they might amplify the inflammatory response of NK cells to levels harmful to the embryo. This amplified inflammatory response could be a self-perpetuating process that eventually results in a miscarriage. Therefore, there is a clear association between endometrial inflammation and miscarriage, where NK cells play a key role in regulating inflammation levels and maintaining pregnancy stability.

INFLAMMATORY CYTOKINES

Inflammatory cytokines play a pivotal role during pregnancy. In a typical pregnancy process, certain inflammatory cytokines aid in the implantation of the embryo and the formation of the placenta. However, when the levels of these factors become imbalanced, it can lead to adverse pregnancy outcomes. Below is a detailed description of the impact of other inflammatory cytokines on pregnancy.

The pro-inflammatory cytokines

The pro-inflammatory cytokines, such as TNF-α, IL-6, and interleukin-1β (IL-1β), are produced during inflammatory processes [25–27]. At normal levels, these factors aid in embryo implantation. However, when their levels become elevated, they can lead to embryo implantation failure, placental dysfunction, and miscarriage. Tumor necrosis factor-alpha has been associated with an increased risk of recurrent miscarriage, especially in individuals with the HLA-DR3 genotype, which is linked to high TNF-α production [28]. Tumor necrosis factor-alpha can not only amplify the inflammatory response, which may adversely affect embryo implantation and growth, but also induce excessive apoptosis of embryo and endometrial cells, leading to embryo implantation failure or early abortion [29]. In addition, TNF-α may also interfere with the blood supply of the endometrium, disrupt the type 1 T helper cells (Th1)/type 2 T helper cells (Th2) balance of immune cells, and change the endometrial environment, making it unfavorable for embryo implantation [30, 31]. Research has shown that in patients who have miscarried, there's a significant increase in the serum levels of TNF-α, IFN-γ, interleukin-2 (IL-2), IL-17A, and IL-17F2 [32]. Furthermore, some women at risk of miscarriage have been treated with TNF-α inhibitors. In these patients, the human chorionic gonadotropin (hCG) trajectory returned to a normal pattern within a week, and the obstetric outcomes were encouraging [33]. Interleukins, as a key group of inflammatory factors, have a significant correlation with recurrent miscarriage. Studies have found that, compared to women with recurrent miscarriages, the cytokines of T-helper cells, especially IL-6, are significantly reduced in these women [34]. This reduced expression pattern is further confirmed in the IL-6 and IL-1α mRNA of the secretory phase endometrium [35]. Moreover, the expression of interleukin-15 (IL-15) and leukemia inhibitory factor in the endometrium of women with recurrent implantation failure after in vitro fertilization (IVF) is related to the number of uNK cells, suggesting their potential role in recurrent miscarriage [36]. Notably, compared to women with idiopathic recurrent miscarriage, there are polymorphisms in the gene

encoding for the Interleukin-1 receptor antagonist, providing new clues for the etiology of miscarriage [37]. Lastly, the significant reduction of interleukin-25 (IL-25) and the upregulation of IL-2 further emphasize the pivotal role of interleukins in recurrent miscarriage [38]. These research findings collectively reveal the central role of interleukins in recurrent miscarriage, providing valuable insights for future therapeutic strategies.

On the other hand, CD16- CD56bright NK cells within the uterus appear to be the primary regulators of inflammation levels at the placental interface, possessing a high capability for cytokine secretion [39]. Specifically, the presence of s-HLA-G in the serum can modulate the cytokine production of uterine lymphocytes, thereby reducing the IFN-γ produced by these lymphocytes [40]. The article also mentions other factors related to inflammation and miscarriage. The role of indoleamine 2,3-dioxygenase (IDO) in human pregnancies requires further elucidation. Indoleamine 2,3-dioxygenase might be associated with the function of placental cells and macrophages, which might prevent a type IV inflammatory response against heterologous antigens on the placenta by producing IDO, targeting T lymphocytes [41]. These inflammatory cytokines may play a pivotal role in both normal and abnormal pregnancies, underscoring the central role of inflammation in miscarriages [42].

The anti-inflammatory cytokines

Anti-inflammatory cytokines, especially IL-10 and TGF-, play a pivotal role in maternal immune regulation, ensuring harmonious coexistence between the mother and the embryo. The establishment of this harmonious relationship is partly attributed to the collaboration of regulatory T cells with IL-10 or TGF-β, working together to enhance maternal immune tolerance, thereby providing a safe growth environment for the embryo [43–45]. This perspective is corroborated by the dominant expression of IL-10 and TGF-β in γδT cells in the human early pregnancy decidua, suggesting their immunoregulatory potential in preventing excessive maternal immune responses to the embryo [46, 47]. However, when this balance is disrupted, as seen in patients with unexplained early recurrent miscarriages, the function of regulatory T cells may be compromised, leading to dysregulation of T helper cells producing interleukin-17 [48]. Furthermore, polymorphisms of cytokine genes associated with miscarriage, such as TNF-α, IFN-γ, TGF-β, IL-6, and IL-10, further highlight the central role of anti-inflammatory cytokines in recurrent miscarriage [49, 50]. Overall, there is a significant correlation between anti-inflammatory cytokines and recurrent miscarriage, and their abnormal expression or dysfunction may lead to an excessive immune system attack on the embryo, increasing the risk of miscarriage [51, 52].

Prostaglandins

Prostaglandins, such as prostaglandin E2 (PGE2), play a pivotal role in normal pregnancies, aiding in embryo implantation and uterine dilation [53, 54]. However, an imbalance in its levels can lead to uterine contractions, potentially triggering preterm birth. Studies have revealed that periodontitis might lead to an abnormal increase in PGE2 levels, which is associated with preterm birth and preeclampsia, suggesting that an imbalance in PGE2 levels might be linked to adverse pregnancy outcomes [55]. Moreover, variations in prostaglandin and hormone levels during embryo implantation, pregnancy, and lactation in women prone to miscarriages further validate the crucial role of prostaglandins during pregnancy [56]. When considering the relationship between inflammation and pregnancy, the role of PGE2 becomes particularly significant, especially in inflammation responses related to pregnancy [53]. More critically, amniotic cavity infections are associated with an increase in PGE2, which might be linked to preterm birth. Lastly, infections by Porphyromonas gingivalis might impact the levels of PGE2, further hinting at its potential role in infection-induced miscarriages [57]. In conclusion, prostaglandins, especially PGE2, have a significant correlation with recurrent miscarriages, and an imbalance in their levels might lead to adverse pregnancy outcomes.

CHANGES IN IMMUNE CELLS

T cells

Immune cells such as macrophages, T cells, and natural killer cells play a pivotal role during pregnancy. Their activation status, quantity, and functional changes may be associated with inflammation and the risk of miscarriage. T cells can produce various cytokines, like leukemia inhibitory factor (LIF) and Th2 cytokines [58]. These factors are crucial in normal pregnancies, but in women with recurrent miscarriages, the production of these factors might be defective [59]. Regulatory T cells (Tregs) are a specific type of T cell that plays a key role in immune regulation. Studies have found that the function of Tregs might be compromised in patients with recurrent miscarriages, especially in their inhibitory effect on T helper cells producing interleukin-17 [60]. In normal pregnancies, both the number and function of Tregs increase, aiding the maternal immune system in accepting the semi-allogeneic embryo [61]. Tregs prevent other immune cells, like effector T cells, from attacking the embryo [62]. Abnormalities in Tregs in recurrent miscarriages, in some women with recurrent miscarriages, the number and/or function of Tregs might be compromised [63]. This suggests that their immune system might not effectively suppress the immune response against the embryo, increasing the risk of miscarriage. Studies have found that the inhibitory effect of Tregs on T helper cells producing interleukin-17 is

compromised in patients with unexplained recurrent miscarriages [48]. Interleukin-17 is a pro-inflammatory cytokine, and excessive interleukin-17 might lead to inflammatory responses affecting embryo implantation and development [64]. T cells not only interact with other T cells but also with other types of immune cells, such as macrophages and natural killer cells [65]. These interactions might influence pregnancy outcomes.

Macrophages

Macrophages are a key immune cell population at the maternal-fetal interface, playing a crucial role in the normal implantation and development of the embryo [66]. However, studies have found that obesity, changes in body mass index (BMI), activation of mediator of IRF3 activation (MITA), and other immune responses might affect the function and number of macrophages [67]. Specifically, abnormal activation and increased numbers of macrophages are associated with recurrent miscarriages. For instance, an increase in the number of macrophages in the endometrium during the mid-luteal phase is associated with an increased risk of miscarriage [68]. Additionally, the overexpression of FasL in macrophages during spontaneous miscarriages might be related to the apoptosis of placental cells [69]. In summary, changes in the function and number of macrophages might be key factors leading to recurrent miscarriages.

Natural killer cells

Natural killer cells within the uterus play a crucial role in embryo implantation and the maintenance of pregnancy [70–72]. However, there are evident alterations in the NK cells within the uterus of patients with recurrent spontaneous miscarriages, and such abnormalities might exert detrimental effects on the normal development of the fetus [72, 73]. In fact, studies have unveiled a close association between elevated levels of NK cells and IVF failures as well as recurrent miscarriages, suggesting that an overactivity or excessive number of NK cells might adversely impact the embryo [23, 74]. Notably, pharmacological interventions, such as the pre-pregnancy use of prednisolone, have been demonstrated to effectively reduce the number of NK cells within the uterus, offering a new strategy for the treatment of recurrent miscarriages [75, 76]. Furthermore, the increased number of NK cells in the uterus of women with recurrent miscarriage may lead to an imbalance of the immune response, which in turn affects the implantation and growth of the embryo [77]. This imbalance may be due to the womb NK cells and other immune cells, such as T cells and macrophages, the interaction between is broken [78, 79]. Study also found that the NK cells in peripheral blood of pregnant women during pregnancy can absorb microRNAs associated with placenta, these microRNAs play A key role in regulating gene expression [80]. Vascular endothelial growth factor C contributes to immune tolerance and enhanced endothelial activity in human uterine NK cells at the maternal-fetal interface [81]. Research has also found that the recurrent spontaneous abortion women and *in vitro* fertilization failure, NK cells on the expression of CD69 and CD161 and CD94 increase [82]. According to a study, NK cells mediate maternal recognition of the trophoblast cells of the placenta through uterine NK cells, a key mechanism ensuring the fetus isn't rejected by the maternal immune system. Firstly, according to a study, NK cells mediate maternal recognition of the trophoblast cells of the placenta through uterine NK cells, a key mechanism ensuring the fetus isn't rejected by the maternal immune system. This mechanism aids the mother in successfully accepting and supporting a semi-allogeneic fetus, thereby avoiding potential immune rejection [83]. Secondly, a study in Endocrine Reviews points out that the activity and number of NK cells in early pregnancy are regulated by hormones, such as progesterone and estrogens [83]. This hormonal regulation might be related to the successful implantation and growth of the embryo, as they can influence the function of NK cells, providing a more favorable environment for the embryo. More importantly, according to a study, uterine NK cells are considered the primary source of angiogenic factors [84]. These growth factors are vital for the remodeling of spiral arteries, ensuring the placenta receives adequate blood supply, supporting embryo growth and development [85]. Furthermore, a study in the FASEB Journal emphasizes that uterine NK cells are also considered key factors initiating the remodeling of spiral arteries [86]. These cells promote the dilation and maturation of spiral arteries by secreting various growth factors and cytokines, ensuring the placenta receives ample blood supply [87]. However, despite considerable interest in the role of uterine NK cells during pregnancy, a study in ScienceDirect suggests that their role during pregnancy might be more complex than previously thought. This implies that more research is needed to delve deeper into the exact role of NK cells during pregnancy [86, 88]. In conclusion, the multifaceted roles of NK cells during pregnancy ensure the healthy development of the embryo and the successful progression of pregnancy, as amply confirmed in numerous studies.

INFECTION

Some chronic or acute infections (such as certain bacterial or viral infections) may lead to an inflammatory response in the uterus, thereby increasing the risk of miscarriage.

Bacterial infection

Chronic endometritis is a long-standing inflammation of the endometrium, which may be caused by various reasons,

including bacterial infections. Studies have found that common bacteria and chlamydia are one of the main causes of this inflammation [89, 90]. This inflammation may affect the uterine environment, making it unfavorable for embryo implantation and growth. In women with recurrent miscarriages, the presence of chronic endometritis can be reliably detected through hysteroscopy [91]. Notably, after antibiotic treatment, the pregnancy outcomes of these women significantly improved, further confirming the association between bacterial infections and recurrent miscarriages [92]. Additionally, bacterial vaginosis is a common vaginal infection, mainly due to an imbalance of the normal vaginal flora [93]. This imbalance may increase the growth of other harmful bacteria, leading to inflammation. Research has found that bacterial vaginosis is not only a risk factor for preterm birth but also a strong risk factor for recurrent miscarriage [94]. This may be because this infection alters the uterine microenvironment, making it unfavorable for embryo implantation and growth. *Chlamydia trachomatis* is a common sexually transmitted bacterium that can cause various reproductive system diseases [95]. Research has found that *Chlamydia trachomatis* infection is associated with miscarriage. This may be because the inflammatory response caused by this bacterial infection is detrimental to embryo implantation and growth [96]. If *Chlamydia trachomatis* infection is detected, appropriate treatment should be administered to prevent recurrent miscarriages. Bacterial or fungal infections may activate certain cells of the immune system, such as macrophages and T cells [97]. This activation may initiate a series of immune events, such as inflammatory responses, which may be detrimental to embryo implantation and growth. This immune response may be one of the reasons for miscarriage.

Viral infection

The relationship between recurrent miscarriage and viral infection has been clearly confirmed in multiple studies. For example, some studies have pointed out that the dysfunction of PR-SET7 in the placenta may be related to various viral infection responses, suggesting that viral infections may affect the health and growth of the embryo. MITA, as a potential therapeutic target for viral infections and virus-related diseases, its relationship with recurrent miscarriage has also been focused on, implying that its functional changes may increase the risk of miscarriage [98]. Moreover, the activity of natural killer cells, especially in the early response to viral infections, is believed to be related to infertility and recurrent miscarriage. This further emphasizes that viral infections may affect the maternal immune response, thereby increasing the risk of miscarriage [99]. Viral infections during pregnancy can lead to adverse outcomes. Specifically, BK virus and recent dengue fever infections have been frequently mentioned in studies and are associated with poor pregnancy outcomes. For instance, one study pointed out that BK virus infection might have a negative impact on pregnancy outcomes, potentially due to the virus directly invading embryonic cells or affecting the maternal immune response [100]. Another study mentioned a correlation between recent dengue fever infections and miscarriages, which might be attributed to maternal inflammation or other physiological changes caused by the virus [101]. These research findings emphasize the significance of viral infections during pregnancy and their potential impacts on both embryonic and maternal health. In addition, viral infection may activate or suppress the maternal immune response, thereby adversely affecting the embryo. Virus-infected cells and free viral particles may have triggered an immune response detrimental to embryo survival [102]. Furthermore, viral infection may also cause maternal inflammation, which may indirectly adversely affect the health and development of the embryo [103]. In addition to viral infection, other factors such as the presence of antithyroid antibodies may also be associated with miscarriage, and these factors may act in conjunction with viral infection to increase the risk of miscarriage [104]. Overall, these studies collectively reveal how viral infections are related to recurrent miscarriage through various mechanisms, including affecting placental function, activating the immune system, and altering the uterine microenvironment [105].

AUTOIMMUNE DISEASES AND RECURRENT MISCARRIAGES

Some autoimmune conditions, notably systemic lupus erythematosus (SLE) and aantiphospholipid antibody syndrome, can intensify the body's inflammatory response, elevating the risk of recurrent miscarriages [106, 107].

The link between SLE and recurrent miscarriages has been firmly established through extensive academic research. Studies indicate that lupus anticoagulants, specific antibodies associated with SLE, have a pronounced correlation with recurrent miscarriages, especially among younger patients [108]. Furthermore, individuals with SLE face heightened risks during pregnancy, including spontaneous miscarriages, stillbirths, and fetal growth restrictions. This may be attributed to the immune system's aggressive response to the embryo in SLE patients [109]. Encouragingly, advancements in medical technology and refined disease management over recent decades have led to a decline in miscarriage rates among SLE patients [110]. Additionally, antiphospholipid antibodies, another set of antibodies linked to SLE, have associations not just with miscarriages and thrombocytopenia, but potentially with cardiac abnormalities as well [111]. This body of evidence underscores the

significant relationship between SLE and recurrent miscarriages, particularly in cases with a positive antiphospholipid antibody profile [112].

In the realm of research on recurrent miscarriages, the relationship between antiphospholipid antibody syndrome and miscarriages has been a focal point [113]. While some studies primarily address isolated miscarriages, they also highlight that recurrent miscarriages can be influenced by a myriad of factors, antiphospholipid antibodies being a notable one [112]. This suggests that aberrant immune responses might play a pivotal role in recurrent miscarriages. A specific study involving 50 women diagnosed with antiphospholipid (APS) syndrome revealed that treatments combined with heparin yielded more pronounced outcomes compared to just using low-dose aspirin [114]. This finding underscores the significance of tailored treatment approaches for antiphospholipid antibody syndrome. A comprehensive systematic review corroborated the distinct relationship between antiphospholipid antibodies and recurrent miscarriages, offering a range of effective therapeutic interventions [115]. Importantly, research has shown that pregnant women with untreated antiphospholipid antibodies face an elevated risk of fetal loss, reinforcing the direct connection between these antibodies and recurrent miscarriages [116]. A holistic review delved into the multifaceted causes of recurrent miscarriages, emphasizing the central role of antiphospholipid antibodies, while also shedding light on other potential risk factors, such as genetic, anatomical, and endocrine anomalies [117]. In summary, the collective findings from these studies present a compelling narrative: antiphospholipid antibody syndrome has a pronounced association with recurrent miscarriages, potentially due to the immune system's maladaptive response to the embryo. The overarching theme is clear — autoimmune disorders can significantly influence the likelihood of recurrent miscarriages. When the immune system inadvertently targets its own tissues and organs, it can have detrimental repercussions on pregnancy. Specific autoimmune reactions, especially antiphospholipid antibody syndrome, have been identified as key contributors to recurrent miscarriages.

LIFESTYLE AND ENVIRONMENTAL FACTORS

Factors such as smoking, obesity, and certain environmental pollutants are associated with increased inflammatory responses and a heightened risk of miscarriage [118]. Lifestyle and environmental elements play a pivotal role in the risk of recurrent miscarriages. According to a study, maternal BMI, smoking habits, and the level of urbanization can elevate oxidative stress in newborns, which is subsequently linked to miscarriage risks. Oxidative stress arises when there's an imbalance between free radicals and antioxidants in the body, leading to cellular damage [119]. Furthermore, habits like smoking and alcohol consumption have been proven to affect ovarian function and the quality of oocytes, thereby amplifying the risk of miscarriage [120, 121]. Environmental pollutants, such as heavy metals and organic pollutants, might elevate miscarriage risks by intensifying inflammatory responses. Notably, air pollution, especially fine particulate matter (PM2.5) and ozone, has been linked to various respiratory and cardiovascular diseases [122]. These pollutants might elevate miscarriage risks by inducing inflammatory responses and oxidative stress. Additionally, the quality of male sperm can also be influenced by environmental factors and lifestyle choices, potentially increasing the risk of miscarriage [123]. These factors might impact reproductive health and miscarriage risks by amplifying oxidative stress and inflammatory responses [124].

TREATMENT STRATEGIES

For recurrent miscarriages related to inflammation, some treatment strategies include the use of anti-inflammatory drugs, immunomodulatory therapies, or anticoagulant treatments. Recurrent miscarriage is a complex medical challenge with a myriad of underlying causes. In recent years, researchers have begun to focus on the role of inflammation in this context. Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen or aspirin have been proven to effectively mitigate inflammatory responses, potentially reducing the risk of miscarriage, as indicate [125, 126]. Additionally, immunoglobulin therapy has been shown to effectively modulate the maternal immune response, further decreasing the risk of miscarriage [127, 128]. For the specific condition of antiphospholipid antibody syndrome, the use of heparin combined with low-dose aspirin as a treatment strategy has been demonstrated to effectively reduce the risk of miscarriage [129, 130]. Moreover, studies related to complex regional pain syndrome have pointed out that the induction of TNF-α and IL-1β might be associated with recurrent miscarriages [131]. Oxidative stress in males, the diagnosis of chronic endometritis, the genetics of recurrent miscarriage, and diseases related to inflammation and coagulation are all linked to recurrent miscarriages [20, 132]. In conclusion, research on treatment strategies for recurrent miscarriages associated with inflammation provides valuable therapeutic methods and directions. However, further research is needed to refine and optimize these treatment strategies.

DISCUSSION

The relationship between inflammation and recurrent miscarriage has emerged as a focal point in the field of reproductive medicine. Changes in the endometrial microenvironment, imbalances in immune cells, infections,

and autoimmune diseases are all linked to inflammation, which can potentially impact embryo implantation and growth, leading to miscarriage. Specifically, an excessive inflammatory response can disrupt the receptive nature of the endometrial environment for embryo implantation. Moreover, lifestyle and environmental factors might exacerbate inflammation, further increasing the risk of miscarriage.

However, our understanding of the specific mechanisms by which inflammation leads to recurrent miscarriage remains limited. Future research should delve deeper into these mechanisms to provide more effective therapeutic strategies for patients. Additionally, considering the influence of lifestyle and environmental factors, preventive strategies are equally vital. By making lifestyle modifications, such as quitting smoking, limiting alcohol intake, and avoiding exposure to harmful environmental factors, the risk of recurrent miscarriage might be reduced.

Looking ahead, as our understanding deepens regarding how the endometrial microenvironment and immune cells interact with inflammation, we anticipate the development of more effective treatments, assisting women at risk of recurrent miscarriage in achieving successful pregnancies.

Article information and declarations

Author contributions

All authors contributed to the study conception and design. The first draft of the manuscript was written by Mengsi Lin and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

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The role of berberine in polycystic ovary syndrome — a summary of knowledge

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a widely prevalent condition that affects approximately 5–10% of women of reproductive age. Although first described in the 18th century, a detailed account of the disease was not provided until Stein and Leventhal's 1935 report. Due to the varied symptomatology of PCOS, treatment must be tailored and often involves using multiple drugs for optimal pharmacotherapy. Berberine, an alkaloid with a longstanding history of use, has gained popularity as a potential treatment option for PCOS. Previous studies have demonstrated that berberine can improve hormonal imbalances by reducing testosterone and FAI, increasing SHBG, and mitigating the clinical symptoms of androgen excess, including hirsutism and acne. Moreover, berberine enhances the therapeutic effects of other drugs commonly used in PCOS, such as metformin and oral contraceptive pills. It is generally well-tolerated with a favourable safety profile. However, further research is warranted to establish conclusive evidence regarding berberine's mechanistic underpinnings, therapeutic potential, and long-term safety as a PCOS treatment modality.

Keywords: berberine; polycystic ovary syndrome; PCOS; hyperandrogenism; androgens; PCOS treatment

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a widely prevalent endocrine disorder, affecting 5–10% of women of reproductive age [1]. The first documented description dates to 1721, when an Italian physician and scientist, Antonio Vallisneri, reported a case of a young, overweight woman suffering from infertility. In his article, he compared the size and morphology of her ovaries to doves' eggs, thereby describing the anatomopathological characteristics of PCOS [2, 3]. Two centuries later, Irving Freiler Stein and Michael Leventhal were the first authors to describe the features of this syndrome in their paper "Amenorrhea associated with bilateral polycystic ovaries" [4]. The article published in 1935 presented reports of seven women complaining of infertility and amenorrhea. The authors identified hallmark clinical and pathological features and discussed the possible mechanism of this syndrome.

Despite extensive observations and clinical evaluations of patients with polycystic ovary syndrome, there was a lack of standardized and widely accepted diagnostic criteria. This was due to the heterogenic clinical presentation and differences in laboratory and imaging studies, which resulted in a need for a more precise definition of the syndrome. In response, the experts at the National Institutes of Health (NIH) proposed two diagnostic criteria for PCOS in 1990: 1) oligo-anovulation and 2) clinical or biochemical signs of hyperandrogenism, which were symptoms commonly seen in patients with PCOS. However, this definition did not include the consistent finding of polycystic morphology in imaging studies, which was later incorporated into the consensus during Rotterdam American Society for Reproductive Medicine/European Society of Human Reproduction and Embryology (ESHRE/ASRM) conference [5, 6]. During the conference, the participants classified patients into four distinct phenotypes based on the presence or absence of symptoms. According to the classification, phenotype A is diagnosed when all three criteria are present, while phenotype B is diagnosed when there is androgen excess and oligo-anovulation. Phenotype C is diagnosed when there is androgen excess and polycystic ovarian morphology. Additionally, phenotype D, which includes only polycystic ovarian morphology and oligo-anovulation, was considered a form of PCOS. This classification has become

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clinically useful and widely accepted in the medical community. However, the lack of hyperandrogenism in phenotype D evoked controversy and is not recognized by the AE-PCOS society [7]. In the report of this society, authors proposed the presence three criteria as constituting PCOS: 1) hyperandrogenism: hirsutism and/or hyperandrogenemia, 2) ovarian dysfunction: Oligo-anovulation and/or polycystic ovaries, 3) exclusion of other androgen excess or related disorders [8].

The difference in phenotypes suggests different clinical courses and subsequent outcomes, such as metabolic and cardiovascular complications. Studies suggest that patients with higher levels of androgens may have a higher risk of developing more severe metabolic and cardiovascular disorders, whereas patients with normal levels of androgens might have a lower risk [9–11]. Dapas et al. [12] furthered the understanding of PCOS phenotypes. The authors analyzed PCOS phenotypes using genotypic methods and clustered patients into reproductive and metabolic subgroups. The reproductive group is characterized by high levels of luteinizing hormone and sex hormone-binding globulin but with normal/low body mass index (BMI) and normal insulin levels. In contrast, the metabolic group has high BMI, glucose, and insulin levels but low luteinizing hormone and sex hormone-binding globulin levels. The study results suggest that these subtypes are biologically relevant because they appear to have distinct genetic architecture. Further investigation into the genetic architecture of PCOS may uncover additional subgroups of patients. Gaining a better understanding of the genetic components of these PCOS subtypes can provide important information about the underlying mechanisms. This knowledge paves the way for the development of tailored therapeutic strategies for patients, ultimately leading to improved patient care [13].

LITERATURE SEARCH AND STUDY SELECTION

A narrative literature review was carried out by searching electronic databases, primarily MEDLINE via PubMed. Our selection criteria focused on systematic reviews, meta-analyses, randomized controlled trials, and prospective observational. To be eligible for inclusion, all studies had to be peer-reviewed and written in English. In vitro studies and animal model research were excluded from our analysis. Additionally, publications and articles for which the full text could not be accessed were also excluded.

The search strategy employed the following query: ((“Polycystic Ovary Syndrome”[MeSH Terms] OR “Polycystic Ovary Syndrome”[Title/Abstract] OR “PCOS”[Title/Abstract]) AND (“Berberine”[MeSH Terms] OR “Berberine”[Title/Abstract])) AND ((“Androgens”[MeSH Terms] OR “Androgens”[Title/Abstract] OR “Androgen”[Title/Abstract] OR “Testosterone”[MeSH Terms] OR “Testosterone”[Title/Abstract]) OR (“Hyperandrogenism”[Title/Abstract] OR “Hyperandrogenemia”[Title/Abstract]))

Our search included all literature related to the use of berberine in reducing androgens in PCOS up until February 2023. Additional relevant articles discovered while reviewing the identified publications were also included in our analysis. In total, 8 studies met the inclusion criteria and were incorporated into the review.

PATHOMECHANISM OF HYPERANDROGENISM IN PCOS

The pathophysiology of hyperandrogenism in PCOS is characterized by several mechanisms, including increased androgen synthesis, decreased sex hormone binding protein concentration, and increased 5α-reductase activity. A disruption in normal ovarian and/or adrenal function leads to excess androgen production, which is a defining feature of PCOS (Fig. 1) [7].

Physiologically, the level of androgens is controlled by the hypothalamic-pituitary-ovarian axis. The hypothalamus secretes in a pulsatile manner the gonadotropin-releasing hormone, which stimulates the pituitary gland to release gonadotropins. Luteinizing hormone (LH) acts on ovarian theca cells interacting with LH receptors and inducing androgen production. Simultaneously, follicle-stimulating hormone (FSH) acts on the granulosa cells of the ovary and converts the androgens formed in theca cells into estradiol, which promotes follicular development.

In PCOS, the balance between androgens and FSH is disrupted, which interferes with follicular development [14]. This is associated with alterations in theca cell function, as well as changes in the pituitary gland's responsiveness to gonadotropin-releasing hormone (GnRH). Consequently, there is an elevated secretion of LH, which stimulates theca cells to produce higher levels of androgens; however, the concentrations of FSH and the conversion of androgens to estradiol remain inadequate. This insufficiency leads to the failure to select a dominant follicle, resulting in chronic anovulation [15]. As a result, PCOS is characterized by the enhanced proliferation of small follicles followed by growth arrest, ultimately leading to the characteristic polycystic morphology.

This observation is supported by the in vitro studies that suggest an intrinsic defect as a cause of excess androgen production and the steroidogenic secretory pattern observed in vivo—the excess production of androgens and insulin results in the premature luteinization of the granulosa cells [7]. Higher androgen hormones' concentration can also result from insulin resistance and hyperinsulinemia, leading to lower sex hormone-binding globulin levels [16]. As a result of those processes, the levels of various androgens in patients with PCOS are elevated, including testosterone,

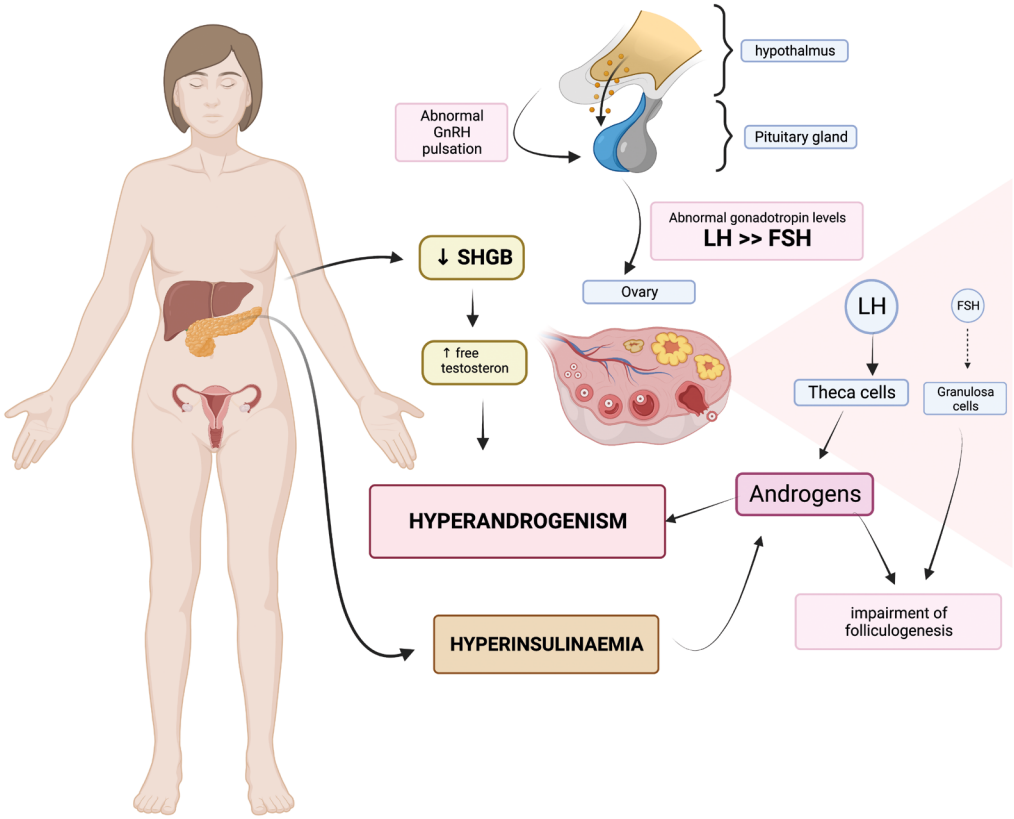

Figure 1. The pathomechanism of androgen excess in polycystic ovary syndrome (PCOS); GnRH — gonadotropin-releasing hormone; LH — luteinizing hormone; FSH — follicle-stimulating hormone; SHGB — sex hormone binding globulin

pro-androgens, androstenedione and dehydroepiandrosterone sulfate (DHEAS) [17]. Over the years, many therapeutics have been proposed to tackle the excess in androgen production. Currently, more and more studies suggest that berberine might be beneficial in the treatment.

To learn more about the management of PCOS and potential therapeutic applications of berberine in PCOS, please see: *Application of berberine in PCOS.*

BERBERINE — A MOLECULAR MECHANISM OF ACTION IN HYPERANDROGENISM

Berberine, a natural alkaloid found in various herbs of the Berberis species, has a long history of medicinal use dating back to ancient times. The fruit of the barberry plant, which contains berberine, was used for its blood-purifying properties as early as 650 BC, as evidenced by clay tablets discovered in the library of the Assyrian emperor Ashurbanipal [18]. Berberine was first isolated and characterized by Buchner and Herberger in 1830, marking the beginning of the modern scientific investigation into this compound [19]. Today, berberine is widely studied for its potential therapeutic applications in various medical conditions. In the latter part of the 20th century, clinical researchers devoted significant efforts to exploring the potential of berberine in addressing diarrheal episodes that arise from various bacterial strains. This line of inquiry led to the development of berberine-based therapies. Today, berberine continues to garner interest from the scientific community for its potential therapeutic applications and historical usage as a natural remedy for various ailments. Investigations have demonstrated that, beyond its longstanding attributed properties, berberine exerts a multifaceted influence on regulating numerous molecular pathways. Studies report evidence of therapeutic use on digestive, cardiovascular, neurological, metabolic, and endocrine diseases [20–32]. Scientists are currently investigating the potential of berberine as a therapeutic agent for the management of hormonal imbalances. It has been established that berberine possesses multifaceted actions that impact various molecular pathways, which have been shown to play an essential role in regulating hormonal levels (Fig. 2). Some studies have also suggested that berberine may influence androgen levels in women with PCOS.

Several studies have shown that berberine can increase sex hormone-binding globulin levels [33–35]. Testosterone is present either in free form or carried by SHBG or albumin. Physiologically, it is mainly bound to sex hormone-binding globulin, and only 1% circulates in its free form. Increasing

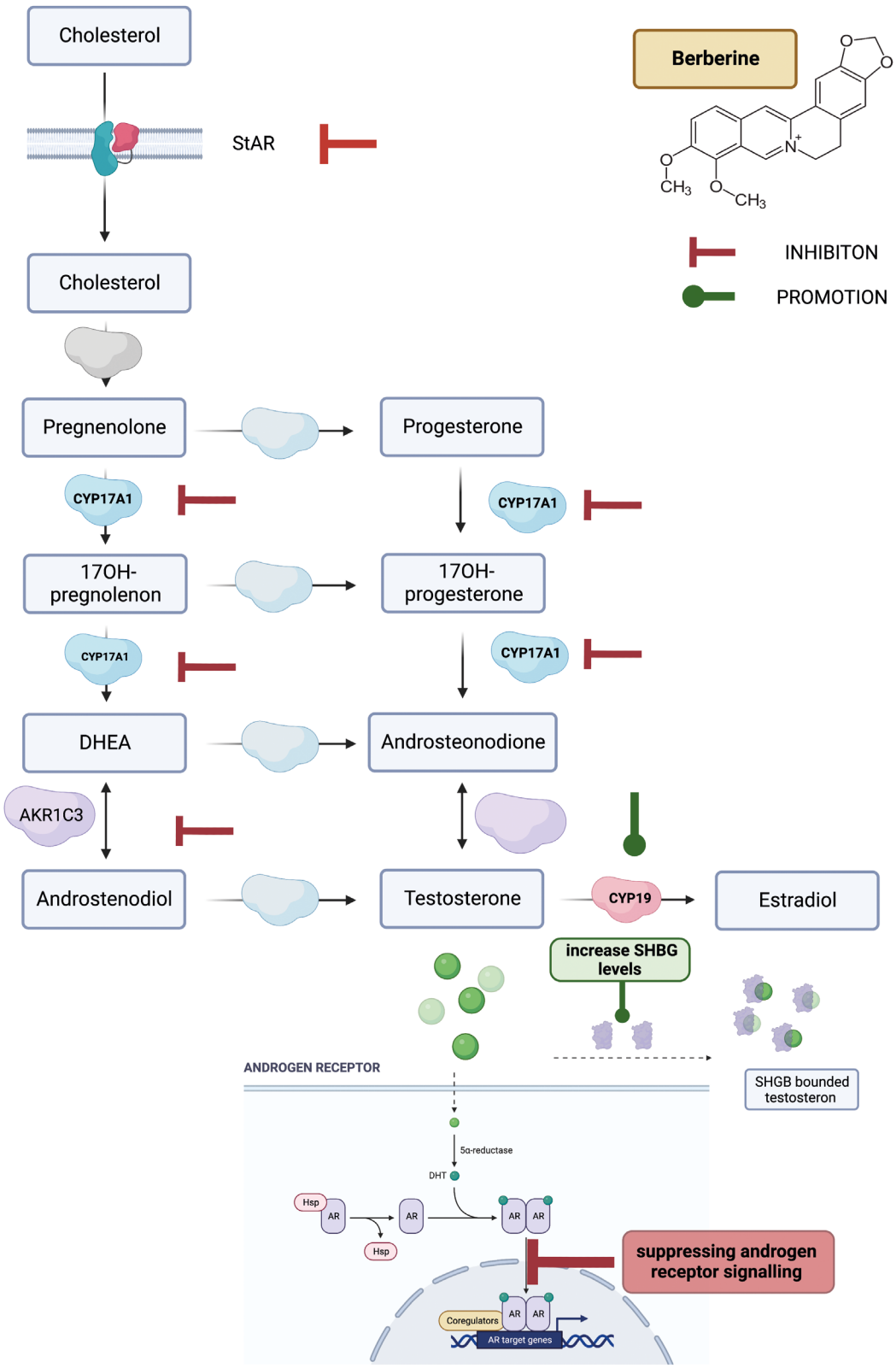

Figure 2. The mechanism of berberine's action in polycystic ovary syndrome (PCOS); StaR — steroidogenic acute regulatory protein; AKR1C3 — aldo--keto reductase 1C3; AR — androgen receptor; SHGB — sex hormone binding globulin

sex hormone-binding globulin levels promotes stability in serum-free androgen levels, decreasing androgen bioavailability.

Moreover, berberine can suppress androgen receptor signalling, leading to an attenuation of androgenic impact. Orio et al. [36] indicated that berberine might exert antiandrogenic properties directly affecting the ovary. The mechanism of this action of berberine remains a matter of discussion. Many researchers have pointed to the various mechanisms. Li et al. [37] found that berberine might promote the degradation of the androgen receptor protein instead of mRNA expression. Another study indicates that activating AMP-activated protein kinase (AMPK) may reduce the number of receptor proteins. Horman et al. [38] also suggested a mechanism for reducing AMPK activity to suppress androgen signalling. Further research to understand this aspect is needed.

Furthermore, the researchers indicate that berberine may cause inhibition of androgen synthesis [39]. Zhang et al. [39] found that berberine acts by decreasing the concentration of steroidogenic acute regulatory protein (StAR). Steroidogenic acute regulatory protein participates in the transportation of cholesterol, which is necessary to produce steroids by theca cells. Steroidogenic acute regulatory protein facilitates the movement of cholesterol across the aqueous space between the outer and inner membranes. Once cholesterol has been transferred, it is then converted into pregnenolone by cytochrome located on the matrix side of the inner mitochondrial membrane. This conversion is essential in providing the necessary precursor for steroid hormone production [40].

Berberine can also reduce the synthesis of androgens inside cells by inhibiting aldo-keto reductase 1C3 (AKR1C3). Inhibiting aldo-keto reductase 1C3 converts inactive hormone precursors into active testosterone and dihydrotestosterone, which are involved in steroid synthesis.

Additionally, berberine can lower the expression of a gene *CYP17a1*, which encodes an enzyme that is involved in conversion of pregnenolone to 17-hydroxypregnenolone and subsequently to dehydroepiandrosterone (DHEA) — an androgen precursor produced in thecal cells. On the other hand, berberine can increase the expression of *CYP19a1*, a gene that encodes aromatase, an enzyme that converts androgens into estrogens [41]. By regulating these genes' expression, berberine may help improve hormonal imbalances and symptoms associated with polycystic ovary syndrome.

THE MANAGEMENT OF HYPERANDROGENISM IN POLYCYSTIC OVARY SYNDROME

Managing polycystic ovary syndrome requires a personalized approach based on the patient's clinical presentation, preferences, and long-term considerations. The pharmacologic treatment of hyperandrogenic symptoms is based on lowering androgen levels and blocking its effects on tissues [42]. Oral contraceptive pills (OCP) are the first-line therapy, as they suppress ovarian androgen production, increase sex hormone-binding globulin, and lower free testosterone levels, thus improving the hormonal profile and alleviating symptoms such as hirsutism and acne. New-generation OCP containing less estrogen have shown effectiveness in treating hyperandrogenism, yet they need further studies to confirm their beneficial effects and long-term safety [43]. Antiandrogens, such as cyproterone acetate, spironolactone, finasteride, and flutamide, are commonly used [42]. Spironolactone is often preferred due to its anti-inflammatory effects and ability to counteract adverse side effects of OCP. However, the use of antiandrogens is limited due to their potential major side effects, which must be carefully considered before prescribing these drugs to patients [44–46]. Metformin is widely used since it improves insulin sensitivity, restores ovarian function, improving the metabolic and hormonal profile [42]. Inositol supplementation is explicitly beneficial in improving the metabolic and hyperandrogenic profile of PCOS women [47]. Inositol also acts as a second messenger of FSH signalling in the ovary, restoring regular menses [47, 48].

Furthermore, the use of incretin mimetics and sodium-glucose co-transporter-2 inhibitors (SGLT2 inhibitors) have been shown to have potential benefits. GLP-1 receptor agonists added to metformin therapy have been found to improve hyperandrogenism and menstrual irregularities and reduce body weight and insulin resistance [49, 50]. In contrast, the effects of SGLT2 inhibitors on hyperandrogenism and menstrual cycles have not been fully explored yet, but they have been found to improve body composition and metabolic parameters in PCOS patients.

APPLICATION OF BERBERINE IN PCOS

Despite the medications referred to previously, there is no panacea for individuals suffering from PCOS. Therefore, researchers expand the armamentarium of drugs used in polycystic ovary syndrome to tackle the plenitude of symptoms presented in this disorder. The historical usage of berberine in traditional medicine and anecdotal reports of its therapeutic potential in reducing androgens prompted the scientific investigation of this plant-based compound in patients with polycystic ovary syndrome.

In a study by Wei et al. [33], one hundred patients with polycystic ovary syndrome and insulin resistance received berberine and metformin treatment as a second-line intervention after the initial first-line treatment involving OCP and lifestyle modifications. The results indicated that both berberine and metformin have a similar impact on androgen

levels, leading to a reduction in testosterone and a corresponding decrease in the free androgen index. The study found no significant difference between these treatments regarding their effect on the patient's hormonal profiles.

An et al. [35] recruited one hundred and fifty infertile women eligible for in vitro fertilization treatment and randomized them into three groups: placebo, metformin, and berberine. In the study, patients receiving either metformin or berberine experienced positive outcomes in reducing their total testosterone levels and free androgen index, as well as increasing their sex hormone-binding globulin concentration and enhancing their carbohydrate metabolism parameters. The study's authors state that the berberine treatment group showed improved weight reduction compared to metformin and placebo. Furthermore, among patients undergoing IVF treatment, berberine led to an improvement in pregnancy rate and a reduction in the risk of ovarian hyperstimulation syndrome.

Rondanelli et al. [1] conducted a study in which twelve patients with PCOS underwent berberine treatment. Their results showed a statistically significant decrease in free testosterone level, free androgen index, and increased sex hormone-binding globulin. Notably, the authors were the first to assess acne status using Global Acne Grading System (GAGS) and the Cardiff Acne Disability Index systems, two widely accepted tools for measuring acne severity and its impact on patients' lives. The improvement in acne status, as evidenced by a reduction from Moderate to Mild in GAGS and from High to Low in CADI, is of significant importance for PCOS patients, as the visible effects of hyperandrogenism can have a negative impact on their mental well-being and various aspects of their lives. These findings suggest that berberine may hold promise as a potential treatment for dermatological pathologies in patients with PCOS.

In the study conducted by Orio et al. [36], fifty women with polycystic ovary syndrome (PCOS) and obesity, experiencing oligomenorrhea, were recruited alongside fifty healthy controls matched for age and BMI. Both groups underwent six months of berberine treatment. The results demonstrated a statistically significant reduction in total testosterone, androstenedione, and free androgen index and a statistically significant increase in sex hormone-binding globulin concentration. Despite these improvements, the values did not reach levels comparable to those of the control group. Furthermore, there were no statistically significant changes in Ferriman-Gallwey score or dehydroepiandrosterone sulfate concentrations after six months of treatment. Although total testosterone, androstenedione, free androgen index, sex hormone-binding globulin concentration, and menses frequency significantly improved after berberine therapy, they did not reach comparable values to controls.

In the study conducted by Mishra et al. [51] on reproductive-aged females with PCOS, the effects of berberine, metformin, and inositol were compared. Participants were randomized into three groups and instructed to maintain their usual lifestyle routines. After 12 weeks of therapy, it was found that all three treatments reduced total testosterone and increased sex hormone-binding globulin. However, there was no significant difference in the mean testosterone values between the three groups. The increase in sex hormone-binding globulin was highest in the group receiving berberine, which was statistically significant compared to those receiving metformin and inositol. Furthermore, a noticeable change in the free androgen index was observed, with the berberine group showing a greater decrease than the inositol group, which was also statistically significant.

In contrast to prior findings, Li et al. [34] reported a reduction in sex hormone-binding globulin levels and no statistically significant effects on hyperandrogenism following the administration of berberine. The authors postulated that the decrease in sex hormone-binding globulin was likely due to the use of oral contraception pills. Nevertheless, Mishra et al. [51] asserted in their discussion that berberine's enhancement of this parameter occurred independently of oral contraception. The findings of their investigation suggest that the combined use of oral contraception pills with berberine yielded a more favourable outcome than oral contraception monotherapy.

Wu et al. [52] conducted a multicenter randomized, double-blinded, placebo-controlled trial to examine the efficacy of berberine, letrozole, and a combination of both treatments in 644 infertile women diagnosed with polycystic ovary syndrome. The primary outcome of the study was cumulative live births. Contrary to the initial hypothesis, the researchers found no evidence to support the superiority of the combined letrozole and berberine treatment over letrozole alone in achieving live births among the participants. Despite the metabolic profile improvements associated with berberine, it did not significantly affect ovulation or live birth rates when combined with letrozole. Moreover, the study did not find notable differences in live birth rates when considering factors such as BMI, hirsutism score, menstrual patterns, and duration of infertility. Wu et al. posited that the observed differences in treatment outcomes might be related to the distinct phenotypes of PCOS in various populations. They noted that a Chinese cohort is more likely to exhibit less hyperandrogenism and leaner body types compared to a European cohort.

Li et al. [53] conducted a systematic review of nine randomized controlled trials to assess the efficacy of berberine and metformin in treating PCOS-related hyperandrogenism and insulin resistance. The authors found significant within-group changes in luteinizing hormone and

testosterone in the berberine group but no significant changes in the metformin group. However, there was no statistically significant difference between the two groups. In two of the nine trials, the authors compared the effectiveness of the combination of berberine and metformin versus metformin alone. They found a statistically significant reduction in luteinizing hormone, LH/ FSH ratio, and testosterone in the berberine and metformin groups. Despite these results, the evidence remains insufficient to confirm the superiority of using the combination of berberine and metformin over metformin alone in improving endocrine indices in women with PCOS.

DOSING, TOLERABILITY, AND SAFETY OF BERBERINE

Various studies on berberine have implemented different dosages and treatment durations. Some researchers, such as Wei and An, administered 500 mg three times a day, with treatment periods ranging from three to six months. Others, including Rondanelli and Li, chose dosages of 550 mg or 400 mg twice a day, lasting for two or four months, respectively [1, 53]. In a different approach, Orio and Mishra both employed a dosage of 500 mg twice a day, with durations of six and three months, respectively. Lastly, Wu used a higher single daily dose of 1500 mg over a six-month period [52]. These varied dosages and therapy durations reflect the diverse approaches taken to investigate the effects of berberine and might affect the adverse effect rates.

The safety and tolerability of berberine have been evaluated in several studies. Rondanelli et al. [54] assessed adverse events in subjects using a particular formulation of berberine and found no observed or reported side effects, particularly gastrointestinal discomfort. Wei [33] reported that subjects in a clinical study tolerated berberine well, and no significant renal or hepatic function changes were observed. However, an overdose can cause side effects such as diarrhoea, constipation, flatulence, and abdominal pain. Orio et al. [36] found that only two patients experienced mild and transient constipation, while compliance was high with no patients discontinuing treatment.

In contrast, metformin, commonly used as an insulin-sensitizing agent for PCOS, has been associated with stomach upset, loss of appetite, and kidney injury [53]. Studies comparing the adverse effects of berberine, and metformin have reported that gastrointestinal adverse reactions were less severe in the berberine group. However, further studies are needed to comprehensively evaluate the adverse effects of berberine in long-term use, especially in young women and early pregnancy.

SUMMARY AND FUTURE DIRECTIONS

Despite its longstanding tradition of use and perceived safety, berberine continues to be classified as a supplement. Its popularity among patients can be attributed to its widespread availability, lack of prescription requirements, and natural origin, which many patients equate with safety and fewer side effects. However, considering the current lack of sufficient scientific evidence regarding its safety and efficacy, it should not be routinely prescribed as a polycystic ovary syndrome treatment. Nevertheless, studies conducted to date indicate that its results can be efficacious, sometimes surpassing those of conventional PCOS medications. Apart from its described androgen-lowering effect, berberine has demonstrated other beneficial effects, such as weight reduction, improved carbohydrate metabolism, improved lipid parameters, regulation of the menstrual cycle, positive effects on fertility, and other protective effects that have been associated with numerous chronic diseases. Despite such wide-ranging therapeutic actions attributed to numerous cellular mechanisms, there is still no comprehensive theory elucidating the molecular mechanism of action of berberine, which is an area of great interest for further investigation in basic research.

Considering the popularity of this supplement, limited data and its many anecdotal therapeutic effects, a larger, multicenter, double-blind, randomized clinical trial investigating the effects of berberine is warranted. As part of the clinical trial, it would be crucial to ascertain the following:

1. What is the long-term safety profile of berberine as a treatment for PCOS?
2. Are there any patient subgroups for which berberine is contraindicated in PCOS treatment?
3. What should potential drug interactions be considered when using berberine with other commonly used medications?
4. What is the optimal dosage of berberine for achieving therapeutic benefits in PCOS treatment?
5. Does berberine exhibit superior therapeutic effects compared to standard drugs used in PCOS treatment?
6. Can berberine be combined with metformin and oral contraceptive pills to enhance their beneficial effects in PCOS treatment?
7. Which patient subgroups may derive the most significant benefit from the use of berberine in PCOS treatment?
8. Does berberine significantly lower androgen levels in PCOS patients comparing to placebo?
9. Does berberine affect reducing symptoms associated with hyperandrogenemia, such as hirsutism, acne, alopecia, and hyperpigmentation?

10. Is the reduction in PCOS symptoms achieved through berberine treatment associated with significantly improving quality of life?
11. Does berberine have a beneficial effect on fertility in patients with excessive androgen levels in PCOS?
12. Can berberine reduce the risk of metabolic and cardiovascular diseases associated with PCOS?

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Conflict of interest

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RECCOMENDATIONS

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Fetal therapy guidelines of the Polish Society of Gynecologists and Obstetricians — Fetal Therapy Section

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INTRODUCTION

The last two decades have witnessed significant changes in the fields of fetal therapy and surgery. Owing to the latest advancements in ultrasound imaging techniques, it is currently possible to diagnose anatomical defects at the initial stages of pregnancy, while miniaturized surgical tools allow to perform increasingly complicated and complex procedures *in utero*.

Evidence-based medicine (EBM) has become a vital aspect of patient management in all surgical specialties. Compared to the results of large — often multicenter and randomized — studies, the impact of the reports and recommendations from individual clinicians has markedly decreased. The value of own experience has diminished when contrasted with the findings of large sample size studies which allow for comprehensive and reliable assessment of fetal anatomical defects and proper eligibility process for intrauterine interventions. Importantly, only those fetuses who will benefit from these procedures should be deemed eligible for surgery. Surgical approach is on a par with justifiable expectant management and

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Table 1. Ultrasonographic criteria for polyhydramnios

Polyhydramnios	Mild	Moderate	Severe
MVP	8.0–11.9 cm	12.0–15.9 cm	≥ 16.0 cm
AFI	24.0–29.9 cm	30.0–34.9 cm	≥ 35.0 cm

MVP — maximum vertical pocket; AFI — amniotic fluid index

the decision to forgo surgery. A medical intervention, defined as 'at least a break in the continuity of the skin', in a non-sick patient *i.e.* the mother of the affected fetus, represents a unique aspect of fetal therapy. The intervention aims at improving fetal prognosis but it may be associated with considerable discomfort and risk for complications in the mother. Therefore, it is crucial for all fetal therapy procedures to be merited and performed by experienced professionals at highly specialized healthcare centers, and only if these interventions might save fetal life or improve the prognosis. Evidence-based consultation, which allows the patient to make an informed decision, and professional psychological care are the two key elements of fetal therapy. It needs to be emphasized that centralized care benefits both, the woman and the child, as it ensures that a high number of intrauterine procedures are performed at a given center, enhancing the skill set and expertise of the medical professionals.

The goal of this guideline is to systematize intrauterine procedures and to recapitulate their applications and recommendations, in accord with the EBM standards. Most of the procedures described in this guideline have been organized by anatomical regions. Amniocentesis and chorionic villous sampling have been purposefully excluded as they are used in invasive prenatal diagnostics and have been extensively discussed elsewhere.

The authors wish to emphasize that this guideline was compiled based on the currently available and up-to-date findings. Future reports and data may change the recommendations presented below.

POLYHYDRAMNIOS

Polyhydramnios is defined as the excess of the amniotic fluid in the uterus and is diagnosed if the amniotic fluid index (AFI) is ≥ 24 cm, or the depth of the maximum vertical pocket (MVP) in the anterior-posterior view is ≥ 8 cm on ultrasound [1]. The prevalence of polyhydramnios has been estimated at 1–2% of all gestations. Polyhydramnios is either idiopathic (40–50%) or caused by congenital fetal defects or maternal diseases.

Fetal defects (including many genetic conditions, neurovascular, gastrointestinal diseases or vascular rings) are more often responsible for the development of severe polyhydramnios, whereas mild polyhydramnios is mostly associated with gestational diabetes, multiple gestation, or Rh incompatibility.

Detailed ultrasound screening and evaluation of the genetic risk factors, especially for trisomy 21 and 18, should constitute the first stages of the diagnostic process for polyhydramnios. There is no premise for routine genetic testing in idiopathic polyhydramnios [2, 3]. Likewise, there is usually no reason to screen for cytomegalovirus (CMV) infections and toxoplasmosis [4, 5]. Irrespective of the fetal evaluation, it is necessary to analyze maternal risk factors, especially to exclude diabetes or Rh incompatibility, and to collect and screen maternal medical history for medicine, substance and drug use [6].

Polyhydramnios can be classified as **mild, moderate and severe** (Tab. 1). Its severity correlates with the risk for the following complications: preterm labor, abnormal fetal presentation, placental abruption, or cord prolapse.

In mild and moderate polyhydramnios, the management typically consists in regular (every 1–2 weeks) monitoring for changes in the amniotic fluid volume (AFV), maternal wellbeing, and risk for preterm delivery. Nevertheless, due to the subjectivity and limitations of ultrasound evaluation of AFV, amnioreduction should always be considered if the pregnant woman reports clinical symptoms of polyhydramnios.

Amnioreduction

1. In severe polyhydramnios, amnioreduction is recommended in case of severe maternal dyspnea or discomfort which interferes with normal daily functioning [1].
2. In asymptomatic patients or those with well-tolerated symptoms and moderate dyspnea, as well as in mild polyhydramnios, amnioreduction is not recommended [1].
3. In patients < 32 weeks gestational age (GA) with uterine contractility present before the procedure, **indomethacin** may be considered before, during, or after amnioreduction, for example at the dose of 4 × 25 mg for 48 hours [1]; reports about short-term effects of using small doses of indomethacin before 32 weeks GA and the risk for premature closure of the arterial duct are conflicting [7–10]; still, it is necessary to take that risk into account when using indomethacin, especially > 3 days and monitor the patency of the arterial duct.
4. If the only goal of therapy is to decrease the volume of the amniotic fluid, **indomethacin** is not recommended in patients without concomitant clinical symptoms of polyhydramnios [1].

5. After amnioreduction, it is essential to monitor AFV every 1–2 weeks, and repeated amnioreduction should be considered if polyhydramnios recurs and if the mother becomes symptomatic.

Procedure

Amnioreduction is typically performed in local anesthesia, using an ultrasound-guided 18-gauge (in certain cases 16) needle. Excess fluid may be evacuated using a 50-mL syringe or continuous suction at 100–125 mL/min. The procedure is continued until AFI of 15–20 cm or MVP of < 8 cm are achieved. The literature offers no clear guidelines about the recommended volume of fluid to be evacuated at one time, but a threshold of max. 2–2.5 Liters is typically advised. A course of steroids and tocolysis may be considered in fetuses under 34 weeks GA. There is no consensus regarding antibiotic prophylaxis [11]. However, it seems prudent to administer prophylactic antibiotics in case of complications during the intervention, the need for repeat puncture of the amniotic sac, or prolonged duration of the procedure.

Possible amnioreduction-related complications

The most common complications include prelabor rupture of the membranes within 48 hours after the procedure (1%), premature labor within 48 hours (4%), intraamniotic infection (< 1%), and placental abruption (< 1%) [11].

The literature offers no evidence that amnioreduction prolongs the duration of pregnancy by reducing the risk for spontaneous premature labor.

Delivery

Timing and mode of delivery should depend on the cause of polyhydramnios or other obstetric indications. In mild and moderate idiopathic polyhydramnios, vaginal delivery is recommended at term, but no later than between 39 + 0 and 40 + 6 weeks GA, whereas in severe polyhydramnios delivery may be considered after 37 weeks GA [1, 12]. During labor, it is vital to monitor fetal position as the excessive amount of fluid — and the associated higher fetal mobility — may promote fetal conversion to transverse lie or breech presentation. Additionally, spontaneous rupture of the membranes may cause sudden severe uterine decompression, resulting in placental separation or cord prolapse. Gradual transabdominal or vaginal amnioreduction may be considered as a prophylactic measure to lower the risk for these complications, on condition the fetal head is positioned adequately.

OLIGOHYDRAMNIOS

The amniotic fluid surrounds the fetus, cushioning it from trauma and providing a safe environment. The fluid is indispensable for fetal development. Oligohydramnios is defined as decreased volume of the amniotic fluid, while the absence of the fluid is known as anhydramnios. Oligohydramnios is diagnosed at AFI of ≤ 5cm or MVP of ≤ 2 cm. The most common pathologies of pregnancy concomitant with oligohydramnios are presented in Table 2 [13].

The prevalence of oligohydramnios has been estimated at 0.5–5.5% of all pregnancies. The diagnosis of oligohydramnios at any stage of pregnancy is a warning sign and may be indicative of a serious threat to fetal wellbeing [13–15]. The etiology and the effects of oligohydramnios on the course of pregnancy and the prognosis depend on the gestational age at which the symptoms of oligohydramnios have been detected on ultrasound. Oligohydramnios in the first trimester is an extremely rare, albeit reported, occurrence and is always associated with unfavorable prognosis for the fetus. In the second trimester, it is mostly caused by defects of the fetal urinary tract (51%) or preterm prelabor rupture of membranes (pPROM) (34%), and in the third trimester by prelabor rupture of membranes (PROM) and fetal growth restriction (FGR) (Tab. 3) [16].

Table 2. Pathologies of pregnancy concomitant with oligohydramnios [13]

Congenital fetal defects, especially of the urinary tract
Prelabor rupture of membranes (PROM)
Fetal growth restriction (FGR)
Twin-to-twin transfusion syndrome (TTTS) (donor)
Post-term pregnancy
Side effects after pharmacotherapy: • prostaglandin synthase inhibitors • angiotensin-converting enzyme inhibitors

Table 3. The most common causes for oligohydramnios, depending on gestational age [16]

I trimester	II trimester	III trimester
• iatrogenic after amniocentesis, CVS • fetal genetic defects, intrauterine fetal demise, amniotic sack rupture • idiopathic (rare), associated with poor fetal prognosis	• urinary tract defects **(obstructive uropathies — 51%)** • **pPROM — 34%** • chorioamniotic membrane separation — 7% • FGR — 5%	• **PROM** • **FGR** • placental separation • use of angiotensin-converting enzyme inhibitors or prostaglandin synthase inhibitors

CVS — chorion villous sampling; pPROM — preterm prelabor rupture of membranes; FGR — fetal growth restriction; PROM — prelabor rupture of membranes

Gestational age at diagnosis affects the fetal prognosis. The diagnosis of oligohydramnios established as early as the second trimester is associated with high rate of unfavorable fetal outcomes. The risk for fetal demise is additionally elevated by congenital defects, genetic malformations, pulmonary hypoplasia, severe prematurity, and intrauterine fetal infection.

In some cases of oligohydramnios (*e.g.* concomitant with early-onset fetal hypotrophy), genetic diagnostics [fetal karyotyping or — preferably — microarray-based comparative genomic hybridization (aCGH) testing] should be considered. However, due to the decreased volume of the amniotic fluid, amniocentesis is not always technically feasible (in such cases cordocentesis should be considered).

Diagnostic amnioinfusion may be considered in some cases of oligohydramnios. The procedure is recommended not only to diagnose the primary defects of the urinary tract, but it may also play a valuable auxiliary role in detecting other concomitant anatomical defects that cannot be diagnosed on ultrasound due to the absence of the amniotic fluid. Diagnostic amnioinfusion may help to differentiate between prelabor rupture of the membranes and urinary tract defects in the fetus, *e.g.* renal agenesis [17].

Continuous therapeutic amnioinfusion in case of pPROM or PROM does not seem to improve the prognosis or lower the risk for intrauterine infection or pulmonary hypoplasia [18–20]. Still, the literature offers casuistic reports about improved prognosis in cases of pPROM or FGR concomitant with oligohydramnios. According to those sources, continuous amnioinfusion (also through the shunt allowing permanent access to the amnion) alleviated cord compression and prolonged pregnancy [21, 22]. At present, the available evidence is not sufficient to support routine infusions, continuous and using the shunt, in such cases. Regardless, it is necessary to emphasize that the final recommendations and eligibility for the amnioinfusion should be issued by an experienced obstetrician-gynecologist or a perinatologist from a tertiary referral center. Reports are scarce due to restricted indications and a small number of such procedures performed globally. One should bear in mind that amnioinfusion may be associated with the risk for hemorrhage, amniotic fluid embolism, and the onset of uterine contractility.

Procedure

Amnioinfusion is typically performed in local anesthesia, using an ultrasound-guided 16- or 18-gauge needle. The fluid (*e.g.* warm Lactated Ringer's solution) is administered using a 50-mL syringe or a rapid infusion set. The procedure is continued until achieving fluid volume which allows for spontaneous and unrestricted fetal movement within the uterus and ultrasound assessment of the fetal anatomy. There is no consensus regarding antibiotic prophylaxis.

NON-IMMUNE HYDROPS FETALIS (NIHF)

Non-immune hydrops fetalis is a pathologic condition characterized by excessive fluid accumulation in at least two interstitial compartments including peritoneal cavity, pleural cavity, pericardium, and skin. The symptoms are frequently accompanied by polyhydramnios and placental edema. The overall prevalence of NIHF has been estimated at 3/10 000 births, although data remain conflicting. The number of NIHF cases diagnosed in the first and second trimester is significantly higher and has been estimated at 1:1600–1:2000 fetuses [23]. The underlying causes for non-immune hydrops fetalis include fetal cardiovascular defects (21.7%), idiopathic etiology (17.8%), genetic factors (13.4%), fetal hematological issues (10.4%), fetal infection (6.7%), fetal chest tumors (6.7%), complications of monochorionic twin pregnancy [twin-to-twin transfusion syndrome (TTTS), twin reversed arterial perfusion (TRAP)] (5.6%), urinary tract defects (2.3%), fetal metabolic diseases (1.1%), and — although rarely — fetal gastrointestinal abnormalities (0.5%) [24–26].

The risk for genetic abnormalities in fetuses with NIHF increases with concomitant structural anomalies or if hydrops fetalis is diagnosed in the first or early in the second trimester. The diagnosis of non-immune hydrops fetalis should be an indication for genetic testing [24]. Microarray-based comparative genomic hybridization remains the method of choice. It detects submicroscopic genomic changes — microdeletions and microduplications, and such genetic issues are found in as many as 7% of the fetuses diagnosed with structural abnormalities and normal karyotype [27, 28]. Importantly, that method does not detect triploidy, which may also present with generalized edema.

After the diagnosis of NIHF, the mother should be referred to a tertiary referral center. As numerous etiologies may lead to non-immune hydrops fetalis, the perinatal care plan should be tailored to the individual needs of the patient. Ultrasound diagnostics, including fetal growth evaluation, diagnosis and monitoring of the existing anomalies, as well as echocardiographic assessment of the anatomy of the fetal heart and circulatory efficiency, are of key importance. Structural anomalies of the fetal heart, vascular anomalies and fetal heart arrhythmias may account for 20% of all NIHF cases [24]. The frequency of follow-up tests needs to be individually adjusted to each patient and should depend on type of abnormality, fetal circulation, the risk for fetal anemia, and the choice of management.

The recommended laboratory tests from maternal blood include complete blood count (CBC), blood typing (AB0 and Rh), indirect Coombs, Kleihauer-Betke, venereal disease research laboratory (VDRL), antibodies against toxoplasmosis and B-19 parvovirus, CMV, anti-RO/SSA antibody, and G6PD test (depending on maternal ethnicity).

Table 4. The recommended range of tests for fetal non-immune hydrops fetalis (NIHF) (depending on the sample)

Maternal blood	Fetal blood	Amniotic fluid	Fetal investigations (*e.g.* pleural effusion, ascitic fluid)
• CBC • blood type (AB0 and Rh) • indirect Coombs • Kleihauer-Betke	• CBC with smear • direct Coombs, • blood type (AB0 and Rh)	• aCGH	• lymphocyte count • total protein • albumin
• VDRL test • antibody test against toxoplasmosis, B-19 parvovirus, cytomegalovirus	• TORCH panel	• PCR for CMV, • PCR for B-19 parvovirus/ /toxoplasmosis	• creatinine/electrolytes
• G6PD	• total protein and albumin		
• anti-RO/SSA antibody	• PCR for CMV • PCR for B-19 parvovirus/ /toxoplasmosis		

CBC — complete blood count; aCGH — microarray-based comparative genomic hybridization; VDRL — venereal disease research laboratory; PCR — polymerase chain reaction; CMV — cytomegalovirus

The recommended laboratory tests from the amniotic fluid include aCGH, polymerase chain reaction (PCR) for CMV, PCR for B-19 parvovirus/toxoplasmosis.

The recommended laboratory tests from fetal blood include CBC with smear, direct Coombs, blood typing (AB0 and Rh), the Toxoplasmosis, Other (Syphilis, Hepatitis B), Rubella, Cytomegalovirus, and Herpes simplex (TORCH) panel, total protein and albumin, PCR for CMV, PCR for B-19 parvovirus/toxoplasmosis.

The recommended laboratory tests from fetal investigations (pleural effusion, ascitic fluid) include lymphocyte count, total protein, albumin, creatinine/electrolytes.

Tests which might be considered for patients with NIHF are presented in Table 4.

Fetal therapy for NIHF

The scope of therapeutic interventions in fetuses with non-immune hydrops fetalis is broad and the choice of optimal therapy depends on the etiology of NIHF and symptom severity. The recommended management may include both, non-invasive and invasive procedures.

Repeated puncture or shunt placement:
— pleural effusion;
— lymphatic system defects (chylothorax);
— ascites.

Administration of the medication to the fetus (cordocentesis):
— hypoalbuminemia (albumins);
— fetal anemia (red cell concentrates).

Delivery

The decision about the timing and mode of delivery, if possible, should be made at a tertiary referral center by an interdisciplinary team, including at least a perinatologist and a neonatologist, ideally a cardiologist/neonatal cardiac surgeon and/or neonatal surgeon. Current obstetric guidelines for preterm delivery (prenatal steroid therapy, the use of magnesium sulfate for neuroprotection) should also be taken into consideration.

Absolute contraindications for the intrauterine procedure

— multiple structural defects;
— severe genetic abnormalities in the fetus;
— mirror (Ballantyne) syndrome in the mother;
— severe preeclampsia in the mother;
— symptoms of progressing intrauterine infection;
— lack of maternal compliance/consent.

Benefits of the intrauterine procedure

The benefits of fetal therapy depend on fetal condition and the principal cause of hydrops. Intrauterine procedures aim to eliminate the direct cause of the fetal defect or to lower the intensity of those symptoms which constituted a threat to fetal wellbeing. Nevertheless, the management of NIHF may in some cases be limited to the treatment of symptoms. The potential benefits should not be outweighed by the risk associated with the procedure.

Complications after the procedure

— pPROM;
— intrauterine infection;
— placental abruption;
— miscarriage, preterm labor;
— transient bleeding from the needle puncture site (cordocentesis);
— cord tamponade (cordocentesis);
— shunt dislocation;
— improper implantation of the shunt;
— intrauterine fetal demise.

FETAL HEMOLYTIC DISEASE

Despite routine antenatal immunoprophylaxis, which consists in administering anti-D immunoglobulin to all non-sensitized, Rh-negative women who present with no anti-D antibodies, alloimmunization to that antigen continues to be the main cause of fetal hemolytic disease.

In fact, all RBC (red blood cells) antigens may trigger alloimmunization. Therefore, other serological conflicts caused by incompatibility with other antigens (*e.g.* C, c and E) and other blood group systems (*e.g.* Kell, MNSs, Kidd, Duffy, Diego, Colton and AB0) should not be excluded. Rh incompatibility and the resulting fetal hemolytic disease constitute a significant issue for perinatal medicine. The prevalence of fetal hemolytic disease has been estimated at 0.2–0.3% of all gestations.

Rh incompatibility means that the maternal immune system produces alloantibodies against fetal antigens. The antibodies in question can cross the placental barrier (active transport), bind to RBC antigens, and cause hemolysis.

It has been estimated that the minimal volume of foreign antigen blood required to sensitize the mother is 0.2 milliliters. Fetomaternal hemorrhage, which leads to alloimmunization, usually occurs in all situations associated with damage to the villi and compromise to the placental barrier, *i.e.* during delivery, miscarriage (spontaneous or induced), surgery for ectopic pregnancy, intrauterine interventions, and during some cases of antenatal hemorrhage.

Over the years, the prognosis for fetuses with hemolytic disease due to Rh incompatibility has significantly improved after the implementation of non-invasive methods of monitoring for fetal anemia, as well as modern and safe methods of intrauterine intravascular blood transfusion. Currently, the survival rate among fetuses with hemolytic disease due to Rh incompatibility after a series of fetal intravascular transfusions has been estimated at 97% [29].

Diagnosis

In cases with low anti-D antibody titer (up to 1:16), expectant management with monthly monitoring of the titer is advised. Fetal ultrasound monitoring is necessary if the titer is elevated. Patients with obstetric history of severe hemolytic disease (intrauterine fetal demise, generalized edema, intrauterine treatment) and those with anti-Kell antibodies are a notable exception. In such cases, ultrasound monitoring should be considered even if the titer is lower (> 4). Ultrasound diagnostics should include Doppler testing of the alloimmunized patients to evaluate the middle cerebral artery peak systolic velocity (MCA PSV) of the fetus, starting from 18 weeks GA. In order for the measurement to be diagnostic, it is necessary to meet the following technical requirements: Doppler angle close to 0°, Doppler gate of 1–2 mm positioned near the Circle of Willis, and light pressure of the transducer on the fetal head. Multiple of median (MoM) of ≥ 1.5 is an indication for cordocentesis and intrauterine treatment [30]. The sensitivity of 86% and specificity of 71% have been confirmed for detecting severe and moderate anemia using MCA PSV in fetuses with no history of transfusions [31].

Fetal genotyping offers yet another non-invasive diagnostic method in cases with Rh incompatibility. It involves isolating cell-free fetal DNA from maternal serum and searching for RBC antigen coding genes, using the real-time polymerase chain reaction (RT-PCR) method. Currently, it is possible to detect the presence of not only the D antigen, but also the remaining antigens of the Rh system and the K antigen of the Kell system. The sensitivity of 99.3% and specificity of 98.4% have been confirmed for such tests in the first and second trimester of pregnancy [32].

Approximately half (54%) of the fetuses whose mothers present with a high antibody titer (over 1:16) will develop the hemolytic disease *in utero* or during infancy. In that group, 26% will develop severe prenatal anemia (fetal edema, intrauterine fetal demise, the need for fetal intrauterine transfusions), 24% will need phototherapy or secondary transfusions after the delivery, and 4% will develop moderate anemia which will require specialist neonatal care [33].

Fetal therapy

Severe fetal anemia — usually caused by Rh incompatibility and the subsequent fetal hemolytic disease — is most often treated with transfusing red cell concentrates into the umbilical cord vessels (cordocentesis) or, in some cases, into the intrahepatic course of the umbilical vein [34]. Fetuses with severe anemia, typically induced by alloimmunization with foreign red blood cell antigens, are eligible for intravascular intrauterine transfusions [35–37]. Fetal anemia due to other causes, *e.g.* parvovirus B19 infection, may also be an indication for a transfusion [36, 38–41]. The volume of the transfused blood depends on gestational age, which has a direct effect on the capacity of the fetal vascular bed. The degree of fetal anemia and the hematologic parameters of the transfused blood should also be taken into consideration. The procedure should be performed by an experienced team, which is able to select the suitable route of transfusion and volume of the RBC concentrate, or else fetal wellbeing and life might be threatened.

Procedure-related complications

The most serious complications after therapeutic cordocentesis (intrauterine transfusion) include [38, 42, 43]:

— transfusion-associated circulatory overload in the fetus after transfusing excess volume of blood;
— umbilical cord occlusion as a result of extravascular blood transfusion – to Wharton's jelly;

— reflex bradycardia;
— intraamniotic hemorrhage;
— intrauterine fetal demise.

Post-procedure monitoring

Intermittent monitoring of the fetal heart is obligatory during the procedure. Periodic monitoring of the fetal heart is advised immediately after the surgery and for a few hours afterwards.

Muscle relaxants and anesthetics may, in some cases, be administered into the fetal circulation pre- and perioperatively. These medicines not only abolish fetal movement but also cause reduced variability on cardiotocography (CTG), which should not affect therapeutic decisions at that time. Abnormal CTG readings are expected for 4–6 hours postoperatively so early cardiotocography is not recommended. CTG monitoring is recommended only if bradycardia, severe tachycardia, or uterine contractility are observed.

BRONCHOPULMONARY SEQUESTRATION (BPS)

Bronchopulmonary sequestration is characterized by the presence of a mass composed of non-functioning lung tissue, with no communication with the tracheobronchial tree. The tumor receives its arterial blood supply from the systemic circulation, most often directly from the descending thoracic aorta (73%), less often from the descending abdominal aorta, celiac artery, or splenic artery (21%). In extremely rare cases, the tumor may be supplied by the right coronary artery or the subclavian artery [44]. The prevalence of BPS has been estimated at 1:15 000 births [45]. On ultrasound, bronchopulmonary sequestration is visualized as hyperechogenic mass in fetal lung tissue, predominantly on the left side, supplied directly by the descending aorta, although other variants are also possible (Fig. 1 and 2). Bronchopulmonary sequestration is intralobar [microcystic congenital pulmonary airway malformation (CPAM)-like presentation] in 75% of the cases, while 25% of the cases are extralobar, with their own pleura and frequently with pleural effusion. The extralobar variant is more often found in fetuses with other concomitant anatomical defects. The use of color Doppler is of key importance in differential diagnosis as it usually allows to identify the source of the blood supply for the tumor. The congenital pulmonary airway malformation volume ratio (CVR) is applied to determine the prognosis. It is calculated using the following formula [46]:

CVR = height × anterior-posterior view × transverse view × 0.52 (constant)/fetal head circumference

Congenital pulmonary airway malformation volume ratio of > 1.6 is associated with a slightly higher risk for hydrops fetalis — as many as 58% of the cases, if the CVR ratio is high [47]. Bronchopulmonary sequestration is rarely concomitant with other chromosomal abnormalities — on its own it is not an indication for invasive diagnostic procedures. Additional structural defects (diaphragmatic hernia, cardiac and spinal defects) may be anticipated in 50% of the cases.

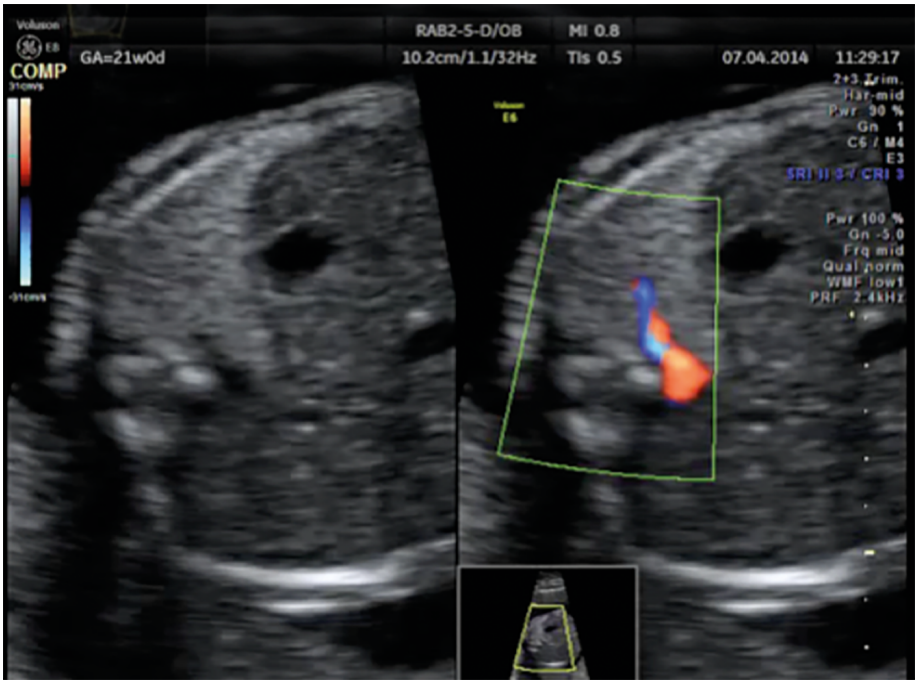

Figure 1. Fetal left lung sequestration and the feeding vessel on color Doppler imaging

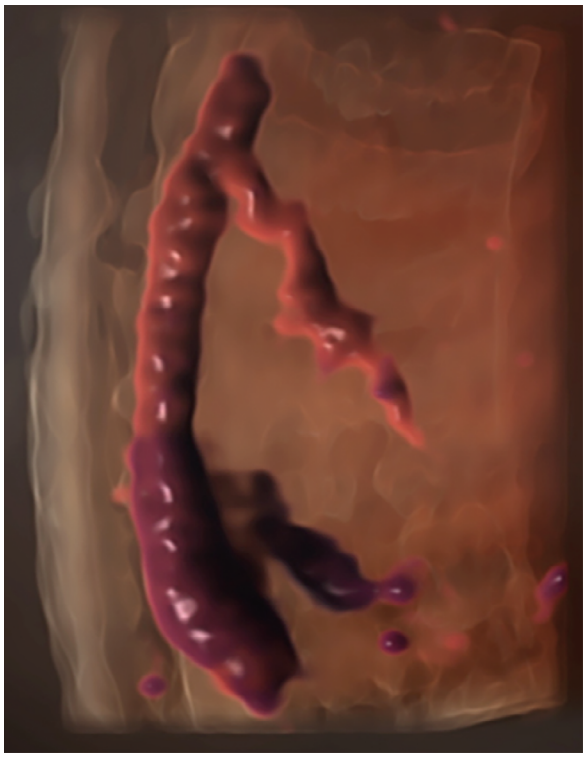

Figure 2. 3D rendering of pulmonary sequestration feeding vessel supplied by the systemic circulation

In BPS, ultrasound monitoring to evaluate tumor growth, pleural effusion and/or fetal edema is recommended every 4 weeks. In 75% of the cases, spontaneous regression and decreased lesion size are observed with progression of pregnancy [48, 49]. Typically, the greatest lesion size is noted between 26–28 weeks GA [50].

Laser coagulation of the tumor-feeding vessel under ultrasound guidance may be considered in the rare cases when BPS is complicated by generalized edema or massive pleural effusion. The procedure requires the operator to have experience with ultrasound-guided surgeries and to know the exact location of the feeding vessel [51]. Possible postoperative complications include preterm labor, PROM, infection, and intrauterine fetal demise.

Uncomplicated bronchopulmonary sequestration is not an indication for a cesarean delivery. In those patients, vaginal delivery is recommended after 38 weeks GA. The prognosis for the fetus is generally (95%) favorable. In cases of BPS complicated by generalized edema, cesarean section at a tertiary referral center is advised.

CONGENITAL PULMONARY AIRWAY MALFORMATION (CPAM)

The prevalence of echostructural abnormalities in fetal lungs presenting as CPAM has been estimated at 1:4000 gestations. Congenital pulmonary airway malformation is a multicystic hamartoma lesion composed of non-functioning lung tissue, predominantly unilateral (> 95%) and restricted to one pulmonary segment or lobe. The blood supply to the lesion comes from the pulmonary circulation. Typically, it is an isolated change, with negligible risk for repeat diagnosis in subsequent pregnancies. Concomitant abnormalities such as cardiac or renal defects as well as tracheoesophageal fistulas are observed in 10% of the cases. After 26 weeks GA, the fetus is at risk for developing polyhydramnios due to the lesion compressing on the fetal esophagus [52, 53].

Congenital pulmonary airway malformation lesions are usually detected during a routine ultrasound test between 18 and 24 weeks GA. Adzick et al. [54], devised an ultrasound classification of CPAM and differentiated between several types of lesions [54]:

- **macrocystic** — single or multiple cysts, at least 5 mm in diameter; intrauterine therapy is possible if symptoms of circulatory failure appear (Fig. 3);
- **microcystic** — solid cysts on ultrasound, less than 5mm in diameter; the prognosis for fetuses with the microcystic type depends on the degree of underdevelopment of the lung tissue and presence of hydrops fetalis (Fig. 4);
- **mixed** — when both CPAM types — microcystic and macrocystic — are detected in the fetus.

The most dynamic growth of CPAM is observed between 18 and 26 weeks GA. The macrocystic tumors are characterized by less dynamic growth as compared to the microcystic lesions. The lesion size decreases with pregnancy progression in approximately 15% of the cases. The CPAM volume ratio (CVR) is a sonographic volumetric index of the mass size, which allows to predict the evolution of the change and undertake adequate diagnostic-therapeutic measures. The index is based on the volume of the cystic mass versus fetal head circumference to adjust the obtained value for gestational age:

CVR = height × anterior-posterior diameter × transverse diameter × 0.52 (constant)/fetal head circumference

Congenital pulmonary airway malformation lesions are associated with elevated risk for developing non-immune hydrops fetalis at CVR above 1.6 [46]. Hydrops fetalis in CPAM is found in <10% of the cases. Typically, if hydrops did not present until 28 weeks GA, the risk of developing it later in pregnancy is extremely low.

Monitoring and delivery

If a CPAM lesion is suspected, ultrasound testing is advised at regular intervals (every 4 weeks at least) to monitor fetal growth, lesion size, and AFV (polyhydramnios may be the result of lesion compression on the fetal esophagus). Due to the altered echogenicity of normal lung tissue early in the third trimester, over 80% of the microcystic changes

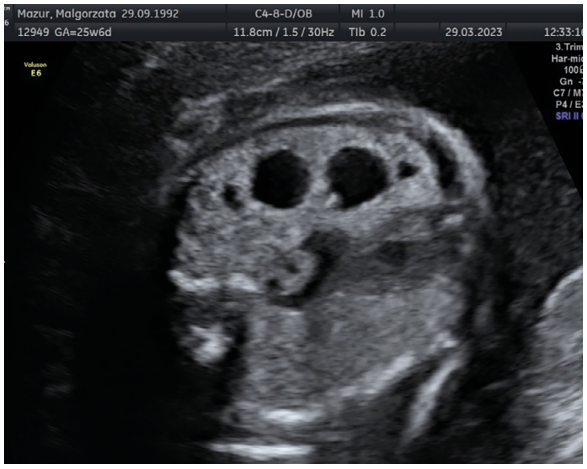

Figure 3. Macrocystic congenital pulmonary airway malformation (CPAM)

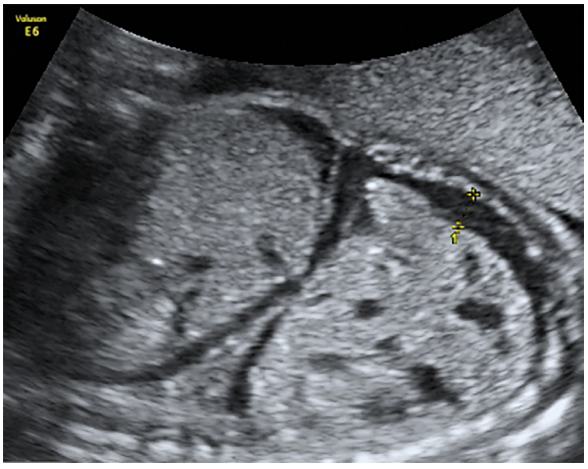

Figure 4. Microcystic congenital pulmonary airway malformation (CPAM)

become less pronounced on ultrasound, although in most cases it is not consistent with lesion regression, but rather technical impossibility to visualize the lesions. Typically, diagnostic imaging after the delivery is necessary [55].

After 38 weeks GA, vaginal delivery at a tertiary referral center — with intense neonatal care unit and neonatal surgery unit on the premises — is recommended. Earlier elective delivery should be considered if signs of fetal growth restriction or circulatory failure have been detected. Intrauterine therapy using thoraco-amniotic shunts may be used if the cystic lesions cause significant mediastinal shift and/or hydrops fetalis. Non-isolated nature of the change is usually an exclusion criterion for intrauterine therapy. The aim of the intrauterine intervention is continuous drainage of the cystic mass allowing to decrease the pressure on the systemic veins and the mediastinal shift, and in consequence to reverse the symptoms of circulatory failure [56, 57].

Risks associated with intrauterine procedure include:

— pain and discomfort at the puncture site;
— shunt dislocation and occlusion which requires reintervention;
— fetal hemorrhage which requires blood transfusion;
— miscarriage or fetal demise (risk 10/100);
— maternal infection (risk < 1/100);
— maternal hemorrhage from the uterine vessels which requires blood transfusion (risk <1/100).

A full course of steroids should be considered in cases with microcystic lesions leading to the development of hydrops fetalis, as some sources claim it decreases tumor volume and leads to the resolution of hydrops [58]. It seems prudent to plan for a steroid therapy in fetuses with severe hydrops and after 32 weeks GA, although the literature lacks consistent reports on the matter. Also, data about sclerotherapy for microcystic lesions and mixed CPAM are scarce [59].

Vaginal delivery is the method of choice after the intrauterine intervention. Immediately after delivery, the shunt needs to be closed or removed from the chest to prevent the development of pneumothorax.

CONGENITAL DIAPHRAGMATIC HERNIA (CDH)

Diaphragmatic hernia is a non-homogenous anatomical defect consistent with varying degrees of herniation of the visceral organs into the thoracic cavity as a result of diaphragmatic discontinuity. Other anatomical abnormalities of the diaphragm include diaphragmatic eventration and complete diaphragmatic agenesis. The prevalence of the defect has been estimated at 1:4000 live births. Posterolateral defects on the left side of the diaphragm comprise most cases of diaphragmatic hernia. The defect is usually unilateral — left-sided in 80% and right-sided in 13% of the cases, with bilateral hernia reported in only 2% of the cases. Congenital diaphragmatic hernia is a non-homogenous anatomical defect, ranging from extremely large defects in the diaphragm, with 90–100% mortality rates, to slight defects of little clinical significance, with 90–100% survival rates among the affected infants [60]. The prognosis depends not only on the size of the defect but also the degree of pulmonary hypoplasia, which in turn depends on the affected side (left or right) and which organs herniated into the thoracic cavity [61]. Hypoplastic lungs in fetuses with CDH are characterized by impaired pulmonary vascular development — over-muscularization and decreased number of pulmonary vessels per lung unit. Also, decreased bronchiolar branching and thickening of the alveolar-capillary barrier are observed. In severe CDH, these abnormalities in the anatomy of the lungs will inhibit effective gas exchange immediately after the cord is cut and will inevitably lead to neonatal death.

In most cases, congenital diaphragmatic hernia is an isolated defect, but concomitant genetic or anatomical

abnormalities (heart or renal defects) have also been reported. Immediately upon diagnosis, it is necessary to exclude the genetic abnormality which is the primary cause of the diaphragmatic defect, especially in cases deemed eligible for an elective intrauterine procedure. The most common abnormalities include trisomy 18 or tetrasomy 12p (Pallister-Killian syndrome) [62]. However, other significantly less common genetic syndromes, with congenital diaphragmatic hernia among their symptoms, have also been reported. Therefore, at least karyotyping is a prudent course of action. It is essential to exclude other anatomical defects in the fetus, especially heart defects, which additionally worsen the prognosis.

Eligibility for the intrauterine procedure

In 1996, Metkus et al. [63], described a sonographic method of assessing CDH severity using the lung area-to-head circumference ratio (LHR), calculated as the lung area opposite the CDH divided by fetal head circumference. A direct correlation has been found between the LHR index and fetal survival, with 0% survival for LHR < 0.6, 61% survival for LHR 0.6–1.35, and 100% survival for LHR > 1.35 [63]. However, the LHR index is not without limitations, chief among them its variability at various stages of the pregnancy. That is why it was necessary to select a parameter which would not depend on the gestational age to such an extent. The observed-to-expected (o/e) LHR, which indirectly evaluates the degree of organ herniation into the chest cavity by measuring the space occupied by the lung (greater protrusions of the fetal organs into the chest cavity corresponds to greater diaphragmatic defect and lower lung volume). The o/e LHR parameter and liver herniation to the fetal chest have been demonstrated to be the most reliable tools of assessing CDH severity and fetal prognosis [64]. The o/e LHR of < 25% is indicative of severe congenital diaphragmatic hernia and constitutes an indication for an intrauterine procedure [65]. A fetus with CDH will be deemed eligible for intrauterine intervention if the following criteria are met: isolated fetal defect, normal fetal karyotype, o/e LHR of < 25%, gestational age of 25–27 weeks, maternal consent.

Fetoscopic endoluminal tracheal occlusion (FETO)

Fetoscopic endoluminal tracheal occlusion is typically performed under epidural anesthesia. After having prepared the surgical field, fetal (presentation, position, location of the mouth) and placental location is determined under ultrasound guidance. The fetus needs to be immobile and anesthetized, which is achieved by intramuscular (lower limb) or intravenous (umbilical vein) medicine administration.

A fetoscope is inserted into the fetal trachea, below the vocal cords, and — with the use of a catheter — a detachable balloon is passed, inflated, and detached to achieve water-tight occlusion (Fig. 5). The balloon is typically placed

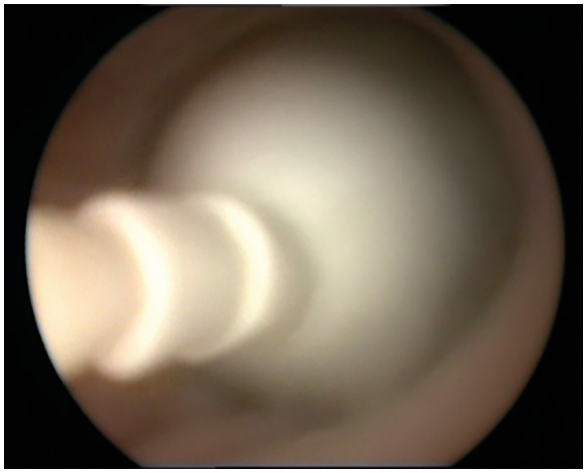

Figure 5. Balloon inside the trachea of a fetus with congenital diaphragmatic hernia (CDH) [fetoscopic endoluminal tracheal occlusion (FETO)]

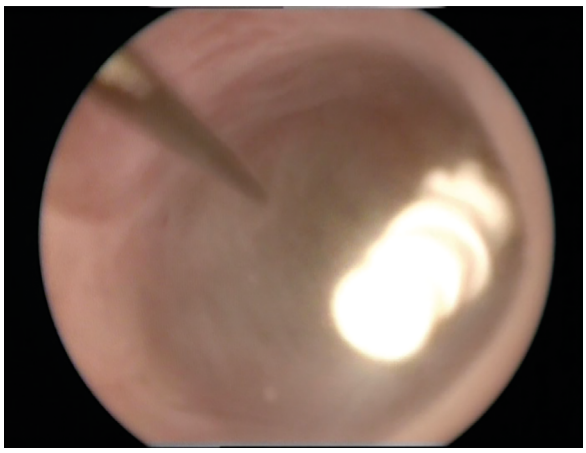

Figure 6. Balloon puncture inside the fetal trachea (visible needle in the upper-left corner)

inside the fetal trachea at 25–27 weeks GA, where it remains until 33–34 weeks. Next, it is punctured with a needle, usually during a second fetoscopic procedure (Fig. 6).

The findings of the randomized TOTAL TRIAL confirmed the efficiency of the FETO procedure in severe congenital diaphragmatic hernia and improved survival, from 15% (no FETO) to 40% (post FETO) [66]. The potential risks associated with the FETO procedure include prelabor rupture of membranes, preterm labor, and placental abruption.

Monitoring and delivery

Fetuses with CDH are at a higher risk for developing polyhydramnios due to the disturbed passage through the fetal gastrointestinal tract. The FETO procedure does not increase the risk for polyhydramnios and, what is more, a timely amnioreduction lowers the risk for preterm labor which might be caused by excess amniotic fluid. Regular

ultrasound monitoring, *i.e.* every 2–4 weeks, is advised. After the FETO procedure, vaginal delivery at the highest level of care center is recommended as CDH is not an indication for cesarean delivery. As severe CDH is associated with unfavorable prognosis, the affected fetuses, especially after the FETO procedure, require multidisciplinary care. Also, transportation of the mother after an intrauterine procedure and the fetus after the delivery should be avoided. The center which provided care to the woman during pregnancy and whose team of perinatologists, neonatologists, and surgeons consulted on the case and may schedule post-delivery procedures on site (intensive care unit [ICU], extracorporeal membrane oxygenation [ECMO], surgery), is the optimal place for delivery.

Emergency balloon puncture

In the event of preterm labor, before elective balloon removal, there are several ways of reversing the tracheal occlusion:

1. An attempt at fetoscopy after membrane rupture — after careful evaluation of the technical conditions and in the absence of regular uterine contractility, it is usually possible to perform amnioinfusion and attempt to remove the balloon using fetoscopy.
2. Balloon rupture through neonatal neck — immediately after delivery, before the cord is cut, a needle is inserted 1–2 cm above the upper sternal region, midline (the procedure may but need not be performed under ultrasound guidance).
3. Balloon rupture through maternal abdomen — it is possible to puncture the balloon by guiding the needle through the maternal abdomen if fetal position allows it. The accessibility depends on fetal presentation and location of the placenta.
4. The EXIT procedure — after neonatal head is delivered and the child is intubated, the balloon is punctured, preferably using a bronchoscope, and the collapsed balloon is removed with small forceps.

Research on developing an improved model of a balloon, which will deflate *in utero* after applying a magnetic field or other triggers, continues and hopefully it will limit the FETO procedure to a single intervention.

SEVERE VENTRICULOMEGALY

Enlargement of the cerebral ventricles of the central nervous system — ventriculomegaly (VM) — is not a separate disease entity in a fetus, but merely a pathological symptom resulting from various causes, chief among them:

— chromosomal abnormalities in the fetus;
— defects of the central nervous system in the fetus;
— intracranial bleeding;
— congenital infection.

Oftentimes, the exact etiology of the condition remains elusive and ventriculomegaly of an idiopathic origin is diagnosed in those cases. In extremely severe cases, VM is associated with elevated risk for perinatal death or unfavorable postnatal outcomes, as well as neurologic defects in the infant.

Enlargement of the lateral cerebral ventricles results in excessive ventricular volume, typically caused by increased pressure of the cerebral spinal fluid secondary to abnormal circulation or abnormal absorption of the CSF. That, in turn, is the source of pressure on the cerebral tissue, leading to irreversible neurological consequences.

Hydrocephalus is defined as increased intracranial pressure in the central nervous system. Antenatal assessment of the intracranial pressure is not feasible, although in extreme cases the effects of the high pressure in the ventricular system may manifest as significantly enlarged head circumference. In the early stage of hydrocephalus, edema, and leukomalacia of the white matter as well as axonal swelling are observed, what later leads to demyelination changes.

Prevalence

The prevalence of mild or moderate ventricular enlargement in the fetal brain has been estimated at 1%, while severe enlargement is observed in approximately 1:1000 of the newborns [67]. The width of the lateral ventricles in the second and third trimester does not usually exceed 10mm, so ventricular thickness of > 10 mm at any stage of pregnancy is defined as ventriculomegaly [68, 69].

Depending on symptom intensity, the following defects have been differentiated:

— **ventriculomegaly**
  Enlargement of the lateral ventricles of the fetal brain with normal biparietal diameter and/or normal circumference of the fetal head. Depending on ventricular enlargement, three stages of ventriculomegaly have been distinguished [70]:
  - mild: 10–12 mm,
  - moderate: 13–15 mm,
  - severe: 15–20 mm;

— **hydrocephalus**
  Hydrocephalus is defined as severe enlargement of the lateral ventricles of the fetal brain and significant enlargement of the biparietal diameter and/or the circumference (at least three standard deviations) of the fetal head. Progressive enlargement of the width and volume of the fetal lateral ventricles during pregnancy is a characteristic symptom of hydrocephalus. The ventricular width is over 20mm and the biparietal diameter of the fetal head (or head circumference) is above 3 standard deviations higher than expected for gestational age. Placement of a ventriculo-amniotic shunt, which allows for continuous evacuation of the excess cerebrospinal

fluid, is one of very few intrauterine therapeutic possibilities in those patients. Still, the literature offers no unambiguous results from large sample size studies to confirm improved neurological prognosis after such interventions. The main outcome and goal of intrauterine therapy in those patients is to decrease the fetal head circumference before delivery by lowering the intracranial pressure. Lowered intracranial pressure may potentially improve the perfusion of the central nervous system (CNS), which in turn may stimulate the reparatory processes in the CNS structures. Nevertheless, the placement of a ventriculo-amniotic shunt does not necessarily lower the risk for neurological damage to the fetus. The primary goal of lowering the pressure in the central nervous system is to slow down the potentially irreversible and destructive changes within the fetal cortex, and to decrease the fetal head circumference before delivery.

Eligibility criteria for the intrauterine procedure

The process of eligibility for invasive diagnostics and intrauterine therapy includes:

— optimal timing for the placement of the ventriculo-amniotic shunt — 23–32 weeks GA;
— fetal karyotyping or, preferably, aCGH testing;
— PCR testing of the amniotic fluid for the following infections:
  - toxoplasmosis,
  - cytomegaly;
— in some cases, the diagnostics of the cerebrospinal fluid obtained during puncture of the enlarged lateral ventricle in the fetal brain (cephalocentesis).

Postnatal management includes

— detailed assessment of the fetal anatomy to exclude concomitant structural anomalies – ultrasound, neurosonography, magnetic resonance imaging;
— monitoring of the lateral ventricular width to determine the dynamics of hydrocephalus progression;
— echocardiography to exclude fetal heart defects;
— maternal serology to detect infection (TORCH);
— if possible and advisable, neurosurgical consultation — to provide information about the type of defect, therapeutic options, and prognosis.

The following criteria need to be met for the fetus to be deemed eligible for intrauterine therapy — implantation of a ventriculo-amniotic shunt

1. Isolated hydrocephalus (lateral ventricle width of ≥ 20 mm and abnormal head circumference) confirmed on ultrasound, neurosonography and/or magnetic resonance imaging (MRI).
2. Dynamic enlargement of the lateral ventricles on subsequent ultrasound tests.
3. Normal karyotype: detection of chromosomal aberrations or presentation with other anatomical defects is indicative of extremely unfavorable prognosis.
4. No evidence of an infection as the underlying cause of the defect.

Prenatal management in the diagnostic-therapeutic process for severe fetal ventriculomegaly/hydrocephalus is presented in Figure 7.

Post-procedure management

During the first few days after the intrauterine intervention, it is necessary to perform an ultrasound test to evaluate the following:

— location of the shunt — normal; possible dislocation into the amniotic sack or into the lateral ventricle of the fetal brain;
— width of the lateral ventricles of the fetal brain;
— minimum and maximum cortical thickness;
— fetal wellbeing.

Delivery after the intrauterine procedure

1. Typically, no indications for earlier elective delivery.
2. Vaginal delivery is possible if fetal head circumference (HC) of < 40cm has been confirmed on ultrasound.
3. Mode and timing of the delivery depend on the recommendation of the obstetric team.

GASTROSCHISIS

Gastroschisis (GS) is a congenital abdominal wall defect, typically located on the right side of the umbilical ring, with the intestine — or other organs, albeit rarely — protruding outside the abdominal cavity. It is a full-thickness defect of the anterior wall of the fetal abdominal cavity, including the peritoneum [71]. The prevalence of gastroschisis has been estimated at 5 in 10000 live births [72, 73]. Typically, it is an isolated defect, and the prevalence of chromosomal abnormalities in fetuses with isolated gastroschisis is similar to that of the general population. Therefore, detection of an isolated defect is not an absolute indication for invasive diagnostics [71, 74–76].

The exact etiology of the defect remains to be elucidated but several theories have been proposed to explain both, the mechanism of its formation and of the secondary damage to the fetal intestine caused by contact with the amniotic fluid. The presence of concomitant intestinal defects (atresia, necrosis, perforation, and torsion) is indicative of a complex gastroschisis (cGS), as compared to simple gastroschisis without any other intestinal abnormalities (sGS). Notably, concomitant defects are more clinically relevant than the pathomechanism of the disease [77].

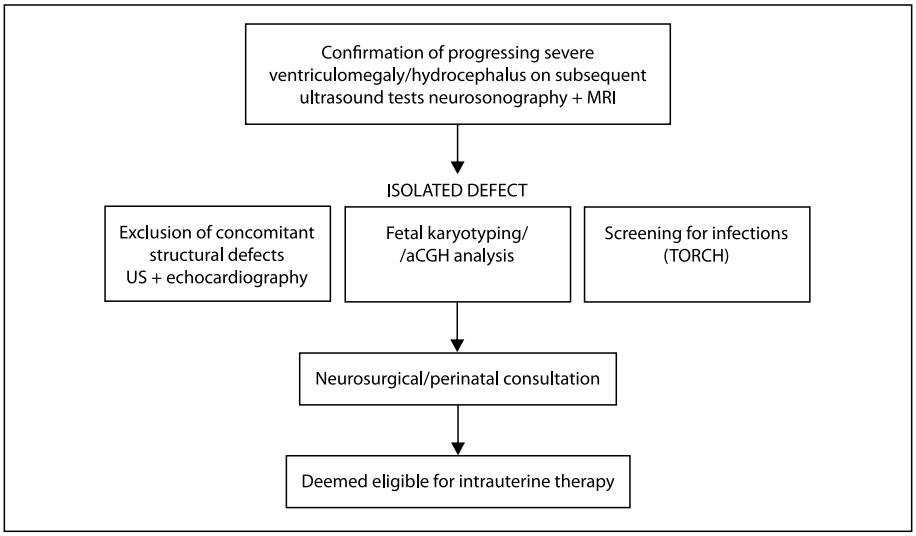

Figure 7. Eligibility stages for intrauterine therapy; MRI — magnetic resonance imaging; aCGH — microarray-based comparative genomic hybridization; TORCH — Toxoplasmosis, Other (Syphilis, Hepatitis B), Rubella, Cytomegalovirus, and Herpes simplex

Overall, cGS is associated with less favorable outcomes [78]. Moreover, progressive intestinal damage — caused by mechanical and chemical stimuli (ischemia, compression from the mesenteric lymph vessels at the site of the defect, and irritants in the amniotic fluid) — is responsible for higher mortality and morbidity also among neonates with sGS [79–81]. Simple gastroschisis in a fetus is associated with favorable prognosis. The neonatal survival rate for fetuses with gastroschisis has been estimated at > 90%, but the rates differ significantly for simple as compared to complex gastroschisis [77, 78, 82–85]. Every single stage of the diagnostic-therapeutic management: from the diagnosis, to proper monitoring, choice of center, time and mode of delivery, duration and type of surgical correction of the defect/surgical intervention, and long-term care, matters as far as improvement of the therapy outcome is concerned [86, 87].

Diagnosis

Prenatal diagnosis of gastroschisis is achieved in 90% of the affected fetuses, in some cases as early as the first trimester. Color Doppler sonography may be used to differentiate between the umbilical loops and the intestinal loops. In the second trimester, it is usually possible to visualize the intestinal defect located on the right side of the umbilical ring and the intestinal loops floating freely in the amniotic fluid. As for differential diagnosis, it is crucial to differentiate between GS and the omphalocele as the diagnostic management of the two conditions varies considerably.

Fetal therapy in gastroschisis

Over the years, amnioexchange has been used in the attempt to lower the concentration of the irritants in the amniotic fluid, which contribute to the inflammatory process. However, randomized studies found that amnioexchange has no definite benefits for fetuses with simple gastroschisis. Serial transabdominal amnioinfusions have also been found to be ineffective in improving the prognosis for the survival or the intestinal and pulmonary function. Nevertheless, amnioinfusion may be propitious for GS fetuses with oligohydramnios [88–91].

The advancements in the field of fetal therapy promote the search for intrauterine therapeutic interventions for complex gastroschisis, but the benefits need to be counterbalanced against the anticipated outcomes and the risk for complications. In theory, antenatal therapy for cGS might prevent secondary damage to the fetal intestine resulting from contact with the irritants in the amniotic fluid or mesenteric ischemia, and in consequence improve the perinatal, neonatal, and long-term outcomes with regard to intrauterine fetal demise, preterm labor, mortality, sepsis, duration of parenteral nutrition and hospitalization, liver failure, number of intestinal complications (short bowel syndrome, necrotizing enterocolitis, functional gastrointestinal disorders), as well as improve the quality of patient life. At present, studies on animal models are being conducted to demonstrate that fetoscopic or open fetal surgery (OFS) enlargement of the defect, with simultaneous covering of the exposed bowel using an artificial graft, might prevent secondary damage to the intestine due to mesenteric ischemia or contact with the irritants in the amniotic fluid. Studies on using transamniotic stem cell therapy to restore bowel function, promote wound healing, and minimize inflammation by stimulating tissue regeneration and direct as well as indirect anti-inflammatory treatment, are also in progress [92–94]. Experimental studies on animal models have demonstrated a possibility of closing

the defect *in utero* using OFS and fetoscopic methods [94]. A report about the first successful fetoscopic repair in a fetus with gastroschisis has been published, but further observational and preferably randomized studies are necessary to evaluate the clinical efficacy of fetal surgery for GS [95]. Attempts have been made to use the Ex Utero Intrapartum Treatment — like (EXIT-like) procedure which involves complete reintroduction of the bowel loops and primary closure of the evisceration during an elective cesarean section before the cord is cut and the first breath is drawn, which prevents significant bowel distention caused by neonatal breathing [93, 96].

All antenatal interventions are associated with an inherent risk for fetal complications and that is why eligibility determination process is crucial, especially if the defect is associated with high survival rate. Fetal therapy is justifiable if significant benefits are to be gained; the primary aim of antenatal therapy for gastroschisis is to prevent secondary bowel injury resulting from contact with the irritants in the amniotic fluid or mesenteric ischemia [94].

Monitoring and delivery

Monitoring of the fetal growth is typically performed at 24 weeks GA and repeated every 3–4 weeks [97]. Most authors recommend more frequent monitoring from 32 weeks GA onwards (every 2 weeks) to evaluate fetal growth, AFI index, and Doppler test. If abnormal fetal growth is confirmed, additional CTG (once a week) is advised [97, 98]. In fetuses with growth restriction or significant bowel distension before 32 weeks GA, monitoring is typically initiated earlier because FGR in fetuses with abdominal defects may be associated with elevated risk for complications, including fetal demise [99, 100].

Timing and mode of delivery remain the topic of much heated debate. The literature lacks conclusive evidence relating to the optimal mode and gestational age at delivery. In the absence of unambiguous data about the effect of continuous exposure of the fetal bowel to the amniotic fluid and the consequences of preterm labor, there is no consensus about the benefits of preterm as compared to term delivery for fetuses with gastroschisis [101, 102]. Nevertheless, delivery at ≥ 38 weeks GA has been reported as more beneficial by a significant number of sources, except for cases associated with a threat to fetal wellbeing, abnormal fetal growth, or significant dilation of the bowel loops.

Considering the above, the choice of the mode of delivery and timing should remain at the discretion of the center providing care to the mother. The decisions concerning gestational age and mode of delivery are made based on the following factors: stage of pregnancy, results of ultrasound tests (fetal growth, AFI, Doppler test, fetal bowel presentation), and CTG. Pre-delivery consultation with a team of specialists, including maternal-fetal medicine expert, neonatologist, neonatal surgeon, and the mother is advised to discuss the details of perinatal management.

Delivery at a tertiary referral center, which is equipped to treat the neonate surgically immediately upon birth, is always advised as it eliminates the necessity to transport the infant with a severe congenital defect. Much evidence indicates that a delivery at a high level of care center is associated with better neonatal outcome as compared to neonates who required transportation [86, 87].

OBSTRUCTIVE UROPATHY

Obstructive uropathy is an anatomical fetal defect which is defined as physiological blockage that inhibits flow of urine from the fetal kidneys to the ureters, bladder, urethra, and the amniotic sack. The prevalence of hydronephrosis due to obstructive uropathy has been estimated at 5–50/1000 fetuses.

Depending on the location of the obstruction, obstructive uropathy results in unilateral or bilateral dilation of one, several or all of the following elements: pelvicalyceal system, ureter, bladder, proximal part of the urethra. Uropathy is classified into lower urinary tract obstruction (LUTO) or upper urinary tract obstruction (UUTO). The prevalence of LUTO has been estimated at 1/2000–4000 fetuses [103–105]. The most common causes of obstructive uropathy include uteropelvic junction obstruction, urethral valve/agenesis, ureterocele, duplex pelvicalyceal systems, cloaca, compression from the neighboring pathological structures, renal tumors/cysts [103, 106].

Diagnosis

Obstructive uropathy is most often diagnosed in the second or third trimester if the presence of hydronephrosis, dilation of at least one ureter, or significantly distended fetal bladder (vesical wall thickness of up to > 2.5 mm) are observed. Amniotic fluid index may be normal or significantly decreased, depending on the type of the defect.

Definitions

1. Hydronephrosis — renal pelvic dilation in the AP plane of > (8)10 mm and/or calyceal dilation of > 2.5 mm and/or dilated/hyperechogenic renal cortex.
2. Ureteral dilation — the ureter is filled with fluid, in advanced cases with haustral folds (megaureter) [106].
3. Distended bladder — bladder sagittal length (in millimeters) above the following value: (Gestational week + 12) [107].

It is necessary to differentiate between obstructive uropathy and other causes of urinary retention, *e.g.* vesicoureteral reflux (typically presenting without bladder wall

distention), or genetic abnormalities, *e.g.* the megacystis, microcolon, intestinal hypoperistalsis (MMIH) syndrome [103, 108, 109].

Indications for invasive diagnostics/ /concomitant genetic abnormalities

— in isolated, typical obstructive uropathies, the risk for chromosomal abnormalities has been estimated at 3–8%;
— in case of concomitant defects and early diagnosis of a distended bladder, the risk for genetic abnormalities is 10–20%;
— the risk for various syndromes [*e.g.*, vertebral defects, anal atresia, cardiac defects, tracheoesophageal fistula, renal anomalies, and limb abnormalities (VACTERL), campomelic dysplasia] is 5–15%;
— invasive diagnostic procedures — although sometimes challenging, *e.g.*, in anhydramnios — are always recommended for patients undergoing elective intrauterine interventions [103, 106, 110–113].

Prenatal management

— depends on the type of the defect (unilateral or bilateral), AFV, gestational age, choice of intrauterine therapy;
— in unilateral uropathy with normal AFV, expectant management with regular ultrasound monitoring (every 4 weeks) is recommended to assess the progression of the defect, function of the contralateral kidney, AFV, and function of other organs at risk for compression from the obstructed structures [114];
— in LUTO with anhydramnios — invasive diagnostics: genetic (ideally aCGH), evaluation of the prognostic parameters from fetal urine sampling; consultation with the mother about the management (pediatric urologist, neonatologist, psychologist); continuation of the expectant management (prenatal hospice) or diagnostic amnioinfusion, or eligibility determination process for intrauterine intervention.

Fetal interventions

— diagnostic-therapeutic amnioinfusion;
— vesicocentesis with prognostic evaluation of the fetal urine;
— serial amnioinfusions (in selected cases);
— vesicoamniotic shunt [104, 111, 113, 115, 116];
— urethroplasty with a balloon catheter in the posterior urethral valve (PUV) (in selected cases) [117];
— cystoscopy (in selected cases) — PUV ablation [118].
— the literature offers a handful of case reports about using nephroamniotic shunting in some patients, including cases complicated by shunt dislocation to the pleural cavity and iatrogenic pleural effusion [119]. In the absence of conclusive evidence about the benefits and risks associated with the procedure, the use of such management in clinical practice is limited. In light of the above, nephroamniotic shunting is not recommended at present.

Eligibility for the procedure [103, 104, 113, 115, 116, 118, 120]

— early (first trimester, early second trimester) LUTO with rapidly progressing destruction of the upper levels of the urinary tract with anhydramnios;
— bilateral obstruction with progressing oligohydramnios;
— significant bilateral vesicoureteral reflux (pseudouropathy) with progressing destruction of the ureters and/or hydronephrosis;
— unilateral, high-intensity obstruction which negatively affects the function of other organs (*e.g.*, circulatory system);
— normal biochemical parameters of fetal urine sampling (Na < 100 mEq/mL, Cl < 90 mEq/mL, osmotic concentration < 210 mOsm/L, Ca < 2 mmol/L, B2 microglobulin < 2 mg/L);
— no other significant concomitant anomalies and other genetic defects in the fetus.

Exclusion criteria

— unilateral uropathy with preserved function in the non-affected, normal AFV and no detrimental effect on the other organs;
— severe subsequent bilateral hydronephrosis with cortical damage (obstructive dysplasia) and/or abnormal biochemical parameters of urine in the subsequent tests (the abovementioned markers above the normal range);
— severe concomitant defects and/or genetic abnormalities in the fetus;
— general infections;
— lack of maternal consent for treatment.

Benefits of the intrauterine procedure

— preserved renal function (complete/partial);
— no/low risk for pulmonary hypoplasia;
— no/low risk for fetal deformations due to anhydramnios, prune-belly syndrome.

Complications after the procedure

— PROM, infection;
— preterm labor;
— 'urinary ascites' due to iatrogenic damage to the vesical wall/distended ureters;
— dislocation of the vesicoureteral shunt (to the amnion, bladder, peritoneum, through the uterine muscle);
— organ damage (mostly bowel, vascular), fetal demise.

Monitoring and delivery

Due to the possibility of dynamic changes in the fetal urinary tract as well as the amniotic fluid volume, monitoring every 3–4 weeks is recommended. More frequent monitoring is advised after fetal therapy interventions — immediately after an invasive procedure. The decision about the timing and mode of delivery is based on several factors, including gestational age and ultrasound test results (fetal growth, presentation of the urinary tract, AFI, fetal Doppler). Surgery for obstructive uropathy is not an absolute indication for a cesarean delivery. Nevertheless, the final decision about the mode of delivery remains at the discretion of the obstetric team. Apart from the basic obstetric criteria, the decision also depends on the prognosis, fetal abdominal circumference (AC/HC), and fetal wellbeing.

MYELOMENINGOCELE

Myelomeningocele (MMC) is a fetal dysraphism of the spinal cord and spinal canal defined as incomplete fusion of the spine and the structures around the spinal cord [121]. Low folate consumption, antiepileptic drugs, diabetes, environmental (elevated temperature during neurulation) and genetic factors promote the development of MMC [122]. Normal progression of MMC is associated with an intrauterine development of Chiari II malformation, presenting as fetal VM, progressive hindbrain herniation, and loss of motor function in the lower extremities, as well as bladder, bowel, and sphincter dysfunction [123].

The defect may have two anatomical presentations:

— open — with hernia sack (meningocele or myelomeningocele) or without hernia sack (myeloschisis);
— closed — the defect in the spinal cord is covered by skin.

The prevalence of the defect has been estimated at 1:2000 births. The survival rate for the first year of neonatal life is 90%, with 75–80% of the affected individuals reaching adulthood [124, 125].

The diagnosis of a bifid spine involves ultrasound imagining of the dysraphism of the vertebral arches, soft tissues, and skin, most often with hernia sack. At present, myelomeningocele is mostly diagnosed during the ultrasound test between 18–22 weeks GA, but in some cases it is possible to visualize the defect as early as during the first trimester ultrasound, not only by evaluating spinal anatomy, but also indirectly by evaluating the intracranial translucency (IT) — an ultrasound assessment of the fourth ventricle and posterior cranial fossa [126, 127]. Second trimester antenatal scan assesses the fetal spine using the sagittal, transverse, and frontal view. It is essential to establish the upper level of the spinal defect, which is defined as the uppermost vertebra with defectively fused ossification centers. Despite the experience of the expert technicians, as well as highly advanced equipment which is currently used for ultrasound testing, the diagnosis of spinal dysraphism may be challenging or altogether impossible. When in doubt or in cases with complex anatomical defects, MRI imaging is recommended. Ultrasound is used to visualize the abnormalities which are characteristic for MMC, including the spine, the central nervous system, and the lower extremities of the fetus (Tab. 5).

Table 5. Three stages of myelomeningocele (MMC) evaluation on ultrasound

	Parameters assessed on ultrasound
Spine	Upper-level defect/spinal dysraphism, signs of tethered spinal cord, hernia sack, placode location, spinal deformity (scoliosis, kyphosis)
Central nervous system (CNS)	Ventriculomegaly (mild, moderate, severe), microcephaly, colpocephaly, banana sign, lemon sign, degree of hindbrain herniation
Lower extremities	Talipes, abnormal motor function

Indications for invasive diagnostics/ /concomitant genetic defects

Intrauterine intervention may be considered in fetuses with an isolated defect, with normal fetal karyotype as the necessary eligibility criterion. It is associated with the fact that approximately 20% of neural tube defects have a genetic component. The most common chromosomal abnormalities as far as spinal dysraphism is concerned include trisomy 18 and 13, and triploidy, but also single-gene abnormalities.

Prenatal management

If MMC is confirmed, non-directive counselling about the possibilities of pre- and postnatal management and referral to a high level of care center which specializes in fetal diagnostics and therapy are advised. Until the patient is transferred to that center, most associations — including the Fetal Medicine Foundation (FMF) — recommend follow-up visits every 4 weeks. Progressive VM is a typical development in spina bifida and is found in 44% of the fetuses before 24 weeks GA, but in 94% of the same fetuses after 24 weeks GA [128, 129]. The 2003–2010 randomized 'Management of Myelomeningocele Study' study (MOMS) analyzed the outcomes of patients who underwent open fetal surgery as compared to a postnatal repair. Randomization was stopped due to ethical concerns. Antenatal repair turned out to be associated with significantly better neurological prognosis for the newborn (unassisted walking: 42% vs 21%), and lower number of ventriculoperitoneal shunts (40% vs 82%) [130, 131].

Table 6. Management of Myelomeningocele Study (MOMS) eligibility criteria for open fetal surgery for myelomeningocele (MMC)

Inclusion criteria	Exclusion criteria
• Gestational age 20 + 0–25 + 6 weeks • Maternal age ≥ 18 years • Ventricular width of the anterior horns of the lateral ventricle < 18 mm • Hindbrain herniation: CM II > 0° • Singleton pregnancy • Normal fetal karyotype • Preserved mobility of the fetal lower extremities • MMC with upper-level defect at ≥ S1	• Fetal defects concomitant to MMC • Type 1 diabetes • Kyphosis > 30° • Cervical incompetence CL < 20 mm • Placenta previa • BMI ≥ 40 kg/m^2 • Rh D alloimmunization and other Rh alloantibodies • Infections: TORCH, HIV, HCV, HBV, active SARS-COV-2 viremia • Uterine anomalies (Müllerian ducts anomalies) • Contraindications to anesthesia • Lack of support from husband/partner • Hypertension, preeclampsia or eclampsia in the current or previous pregnancy • Epilepsy • Extremely low social-economic status • Lack of consent to long-term hospitalization during the postoperative period • Inadequate patient comprehension of the management • History of > 2 cesarean sections • Uterine myomas • History of pelvic laparotomy with purulent peritonitis

CM — cytomegalovirus; CL — cervical length ; BMI — body mass index; TORCH — Toxoplasmosis, Other (Syphilis, Hepatitis B), Rubella, Cytomegalovirus (CMV), and Herpes simplex

In light of the above, it was concluded that surgical intervention in a fetus with spinal dysraphism *in utero* may improve the neonatal outcome and lower the number of complications associated with the CNS defects and the need for ventriculoperitoneal shunting.

Open fetal surgery

Open fetal surgery (OFS) involves incision of the uterine muscle and positioning the fetus so that the repair of fetal MMC may be performed. The procedure has a neurosurgical status since complete untethering of the spinal cord and anatomic reconstruction may be achieved [132]. Management of Myelomeningocele Study eligibility criteria for OFS are presented in Table 6 [130].

In 2017, the Perinatal Center in Bytom, Poland, reported the following results: better psycho-motor function, decreased risk for postnatal implantation of the ventriculoperitoneal shunts (up to 27.8% in the OFS group vs 80% in the postnatal repair group), as well as lower risk for progression of hindbrain herniation (11% vs 70%) [133]. Intrauterine repair not only mechanically shields the spinal cord from the detrimental effects of the amniotic fluid, but it also reduces inflammatory infiltration within the dura matter and the skin [134]. Another benefit of the intrauterine repair is improved continence and the so-called 'social continence' at 3 years of age, which was achieved in 81% of OFS patients vs 70% in the postnatal repair group [135].

Fetoscopic method

The fetoscopic approach offers an alternative to the laparotomic repair of the spinal dysraphism. It uses a minimally-invasive access to the amniotic cavity, *i.e.*, the entire procedure is performed with tools introduced through the trocars. Therefore, difficulty with trocar placement in the amniotic cavity, for example in very obese patients, is the main contraindication for fetoscopic surgery. One of the trocars is the optic trocar, the remaining ones are used to insert the miniatured tools. After the trocars are inserted into the amniotic cavity, it is insufflated with heated and humidified CO_2. Despite being more technically challenging and time-consuming as compared to the open surgery, the fetoscopic repair is infinitely less invasive for the expectant mother, allowing for shorter convalescence and hospitalization, and decreasing the risk for thromboembolic complications [136]. Eligibility criteria for a fetoscopic repair are presented in Table 7. Benefits of the fetoscopic intervention for MMC as well as preoperative management are presented in Table 8.

Hybrid method

Intrauterine surgery for MMC using the hybrid (Belfort) method offers an interesting alternative to the two techniques for spina bifida repair which had been used so far — open fetal surgery and fully percutaneous fetoscopic repair. It is known as 'the hybrid method' as it combines the elements of the abovementioned surgical techniques. It is also called the 'open fetoscopy' method. The abdominal cavity is opened to exteriorize the uterus (that part of the surgery is identical to the classic OFS), and then the trocars are inserted into the uterus directly through the uterine wall — initiating the fetoscopic phase of the surgery [137].

Clinical observations seem to indicate that this surgical method is beneficial, both in terms of technical aspects as well as complications and patient safety [138]. The main technical limitation of the fully percutaneous fetoscopic method is placental location on the anterior wall, which often inhibits safe placement of the trocars into the uterine cavity and constitutes an exclusion criterion for the procedure. The Belfort technique circumvents that problem as the trocars can be inserted at any place, once the

Table 7. Eligibility criteria for fetoscopic myelomeningocele (MMC) repair

Inclusion criteria	Exclusion criteria
• isolated open spinal dysraphism at Th 1–S 1 • gestational age 24–28 weeks • ventriculomegaly < 18 mm • normal fetal karyotype • cerebral manifestations of spinal dysraphism (hindbrain herniation to the spinal canal)	• cervical length on ultrasound < 20 mm • active HIV, HBV and HCV infection • multiple gestation • placental previa • complete paralysis of the fetal lower extremities • fetal kyphosis > 30° • maternal BMI > 35 kg/m^2 • maternal diseases which increase the risk for complications (uncontrolled diabetes, poorly controlled hypertension, or others)

BMI — body mass index

Table 8. Benefits of the intrauterine fetoscopic intervention for myelomeningocele (MMC) as compared to open fetal surgery and postoperative management

Benefits of intrauterine fetoscopic repair for MMC	• shorter maternal convalescence after surgery as compared to other methods (shorter hospitalization) • lower risk for uterine dehiscence /rupture as compared to the open surgery method • chance for a vaginal delivery
Postoperative management	• hospitalization for 7 days postoperatively • out-patient check-up every 2–4 weeks • follow-up ultrasound testing every 2–4 weeks (assessment of the cerebral manifestations, limb mobility, amniotic fluid volume, fetal growth) • monitoring of the inflammatory markers once a week for the first postoperative month

uterus has been exteriorized, which allows to by-pass the placenta. Another advantage over the open method is that the hybrid method does not require the uterine wall to be excised as the minimally invasive fetoscopic technique is applied once the uterus is exteriorized. Importantly, in the original Belfort technique two trocars are inserted into the uterus and not three, as is usually the case in the fully percutaneous method. Another benefit of this technique is the use of additional supporting sutures, which are placed at the designated trocar sites, thus lowering the risk for amniotic membrane dissection — similar sutures are used by some of the centers offering the fully percutaneous repair. Additionally, it is possible to suture the muscular layer of the uterine wall at the trocar site. Both these elements of the procedure significantly lower the risk for PROM, which is one of the complications after intrauterine interventions [139]. Unlike in case of the open surgery, another advantage of both, intrauterine surgery and fully percutaneous fetoscopic intervention is the possibility of a vaginal delivery. Undoubtedly, this surgical method should be considered in the eligibility determination process for an intrauterine intervention in fetuses with spinal dysraphism.

Benefits of the intrauterine surgery for MMC

According to the available sources, despite significant differences between various surgical techniques, the benefits of intrauterine surgery for MMC in fetuses at 12 months of follow-up are similar [136]:

— minimization of the detrimental effect of the amniotic fluid on the exposed neural tissue;
— lower risk for the necessity of ventriculoperitoneal shunting in the neonate (from 82% to 43%);
— higher chance for unassisted walking (by approximately 50%);
— lower risk for hindbrain herniation (> 90%).

The current trend to modify the surgical techniques is the consequence of the attempts to recreate the stages of a postnatal repair, resulting in better neonatal outcomes. Maternal complications — mostly associated with scar dehiscence — and the possibility of vaginal delivery remain the main differences between the procedures. Reports about the decreasing risk for preterm labor and PROM, especially in case of the hybrid method, are optimistic. Nevertheless, further observational studies, preferably randomized, are necessary to conclusively determine the superiority of one method over the other. The final decision about the surgical method should remain at the discretion of the fetal therapy team. That is why it is vital for the centers which offer different surgical techniques to cooperate, to jointly participate in the eligibility determination process, and even refer the patient to the center which has more experience in the selected method.

Delivery

Fetal MMC is not an indication for cesarean section, although such mode of delivery should be considered in cases with large open defects which include several vertebrae and/or large hernia sack, and/or hydrocephalus, which might be an obstetric challenge. As far as patients undergoing OFS are concerned, cesarean section is advised due to the insufficient amount of time for the hysterotomy site to heal. Vaginal delivery remains an option in case of fetoscopic (0% uterine rupture) and hybrid procedures [136].

Complications

Intrauterine surgery, like all surgical interventions, is associated with the risk for complications, with preterm labor as the most common complication. According to MOMS, preterm labor was observed in 79% of the fetuses from the OFS group, out of those 13% were delivered before 30 weeks GA and 21% reached > 36 weeks GA at delivery [130]. According to the Bytom Clinic data, hysterotomy using a diode laser and uterine muscle suture, combined with the tocolysis protocol and the perioperative exchange of the amniotic fluid, resulted in complete reduction of deliveries at < 30 weeks GA and high rate (36%) of deliveries at > 36 weeks GA [140]. Complications after intrauterine interventions due to fetal MMC, regardless of the surgical techniques, are as follows:

— placental abruption;
— prelabor rupture of the membranes;
— hemorrhage;
— preterm labor;
— intrauterine infection;
— fetal demise.

SACROCOCCYGEAL TERATOMA (SCT)

Sacrococcygeal teratoma (SCT) is a neoplasm which originates from the cells from one, two, or three germ layers: ectoderm, mesoderm, and endoderm. Typically, the tumor is located along the midline of the body, with the sacral region (SCT), neck, and the oropharyngeal cavity (where it is known as the 'epignathus') among the most common locations. Less frequent locations include the brain, pericardium, mediastinum, abdomen, and testicles. The tumor is a rare finding in multiple gestations. SCT is the most frequent tumor in the fetus and the neonate, with the prevalence ranging from 1 in 23 000 to 1 in 40 000 live births. The odds of SCT development are 4-fold higher in female fetuses. Intrauterine fetal demise due to SCT significantly lowers the prevalence of the defect in live birth. Most gestations with SCT require careful obstetric monitoring but are otherwise uncomplicated. Fetal anemia or fetal circulatory failure due to rich tumor vascularization may develop in some cases. Expectant management will typically lead to polyhydramnios, generalized edema with the mirror syndrome in the mother, and even intrauterine fetal death. Polyhydramnios may be the cause of preterm labor. Middle cerebral artery peak systolic velocity may be used as a non-invasive method of screening for fetal anemia.

The diagnosis is usually made in the second or third trimester of pregnancy, if on ultrasound the tumor presents as a mass with mixed echogenicity, partially cystic and partially solid parts, calcifications, and variable perfusion. Approximately 15% of the cases are cystic, the remaining 85% are solid and mixed lesions.

According to the American Academy of Pediatrics Surgical Section (AAPSS) classification, four types of sacrococcygeal teratomas may be distinguished:

— type I — the lesion is almost completely extrapelvic, with only a small part inside the fetal body (47%);
— type II — the lesion is predominantly extrapelvic, with a significant part of the tumor located inside the body (34%);
— type III — most of the lesion is intrapelvic, with only a part of the tumor growing outside the body (9%);
— type IV — the lesion is completely intrapelvic (10%).

An overwhelming majority (80%) of SCT cases are type I and II. Type IV presents the greatest diagnostic challenge, which impedes early diagnosis, and the prognosis is typically unfavorable. The lesions develop inside the uterus, compressing the neighboring organs and leading to ureter or bladder obstruction, and hydronephrosis. MRI testing is advised in such cases.

Approximately 15% of SCT patients present with concomitant congenital defects such as rectal atresia, sacral bone defects, bicornuate vagina and/or uterus, spinal dysraphism, myelomeningocele. Teratomas are mostly sporadic, although the literature offers reports of familial cases, *e.g.*, Currarino syndrome (anorectal anomalies, sacral tumors, sacral bone deformities). Only a handful of SCT cases with concurrent chromosomal aberrations have been reported. Currently, there are no indications for fetal karyotyping in fetuses with SCT, although karyotyping may be used as an eligibility criterion for an intrauterine intervention.

It is essential to diagnose SCT antenatally. In a study of 97 SCT cases in Japan, between 2000 and 2009, the perinatal mortality rate was 26%. Out of those, about 21% were born before 32 weeks GA and the mortality rate in that subgroup was 44%. Fetal demise is mainly observed in cases with a rapidly growing, solid, and highly vascularized tumor, as that quickly leads to circulatory failure in the fetuses with non-immune hydrops fetalis. It is the consequence of the so-called 'vascular steal' phenomenon by the tumor, which mirrors the features of a large arteriovenous malformation. Small tumors (< 10cm) constitute a small risk for the fetus and do not require high-intensity ultrasound monitoring (every 2–3 weeks). Larger and more vascularized tumors should be monitored more frequently (every 7–14 days). Ultrasound testing is used to assess tumor size, AFV, echocardiographic and Doppler evaluation of the circulatory system function, and tumor vascularization.

The tumor may also cause damage to the pelvic structures, with some defects developing *in utero* and others due to surgical resection. Vesical rupture *in utero* and urinary tract occlusion have been reported. Also, problems with the rectal and the urinary tract function may be more

prevalent if a sizable portion of the tumor is located within the fetal pelvis.

Early prognostic classification of fetuses with SCT establishes the tumor volume to fetal weight ratio (TFR). Fetuses with TFR of ≤ 0.12 calculated before 24 weeks GA have more favorable prognosis. TFR of > 0.12 is associated with higher incidence of fetal edema (80%) and mortality rate (60%). In one study, TFR of < 0.12 was linked with a 100% survival rate [141]. Other multicenter case reviews confirmed the correlation between TFR of > 0.12 and poor prognosis. Apart from the TFR index, a series of analyzed cases demonstrated that cystic teratomas were associated with better perinatal outcomes as compared to solid lesions [142].

Fetal therapy

After the fetus is diagnosed with SCT, the mother should be referred to a high level of care center which specializes in intrauterine therapy for full-scale diagnostics and eligibility determination process for *in utero* repair. Ideally, the diagnostic process should be conducted at a center which is equipped to perform the intrauterine intervention. The diagnostic process includes 2D and 3D ultrasound, Doppler, and MRI - if necessary. Doppler test and echocardiography are advised to evaluate fetal circulatory efficiency.

Intrauterine interventions in fetuses with SCT

First attempts at fetal therapy involved tumor resection using either laparotomy or open hysterotomy. At present, minimally invasive techniques are more often advised, including:

— interstitial tumor ablation using laser or radiofrequency;
— vascular laser coagulation of the tumor vessels;
— sclerotherapy of tumor vasculature.

The survival rate was 55% (6/11) for open fetal surgical resection as compared to 30% (6/20) for minimally invasive procedures, including electrosurgery, radiofrequency, and laser ablation. Notably, even though the survival rates were relatively low in both groups (OFS and minimally invasive procedures), the surgery was performed in fetuses with non-immune hydrops fetalis, which is associated with extremely high mortality rates even without intrauterine intervention. Mean gestational age at delivery was < 30 weeks in both groups, which emphasizes the risk for preterm labor after surgical intervention and the need for intensive neonatal care after birth.

In another study, laser interstitial tumor ablation (whose objective is to directly ablate the tumor) and vascular laser coagulation (whose objective is to target the tumor's feeding vessel) were compared. Vascular laser coagulation was performed in 11 fetuses and the survival rate was 63.6%. This outcome was more beneficial as compared to the 40.9% survival rate in 22 fetuses who underwent laser interstitial tumor ablation. The authors hypothesized that sudden tumor necrosis and later risk for hemorrhage contributed to the lower survival rate in case of laser interstitial tumor ablation [143–145]. Intrauterine interventions also include amnioreduction, treatment of fetal anemia, and percutaneous shunting of a secondary obstruction in the fetal urinary tract [146] but clinical experience remains limited.

Eligibility determination process for intrauterine intervention

The process of eligibility determination for intrauterine interventions should take place at a high level of care centers, with considerable experience in fetal therapy. At present, non-immune hydrops fetalis and other symptoms of circulatory failure (*e.g.*, cardiomegaly) are among the most significant eligibility criteria for *in utero* interventions. Ideally, the procedure should be performed between 23 and 30 weeks GA. In case of polyhydramnios or fetal anemia, amnioreduction and intrauterine transfusion are also advised.

The main exclusion criteria for fetal therapy are as follows:

— tumor volume: lesion size of up to 10cm is an indication for expectant management;
— type of change: expectant management is typically recommended for cystic and fluid-filled lesions.

In cases with fetal heart failure after 30 weeks GA, elective cesarean section (after a full-course steroid therapy) and subsequent neonatal surgery might be a better solution and help to avoid intrauterine fetal demise. The survival rate for such a course of action is almost 50%.

Postoperative complications

The most common complications associated with fetal therapy for SCT include preterm labor, tumor rupture and hemorrhage (also during the neonatal period), fetal cardiac arrest, tumor recurrence.

Delivery

In the absence of concomitant abnormalities, without the risk of obstructed labor and with the largest tumor volume of < 10 cm, vaginal delivery may be considered. In the remaining cases, elective cesarean section is recommended, especially after fetal therapy interventions.

MONOCHORIONIC PREGNANCY COMPLICATIONS

Twin-to-twin transfusion syndrome

Twin-to-twin transfusion syndrome is a common complication in a monochorionic pregnancy when at least two fetuses share a placenta. Twin-to-twin transfusion syndrome is a hemodynamic volume imbalance across the vascular

Table 9. The Quintero Staging System for twin-to-twin transfusion syndrome (TTTS)

Stage	I	II	III	IV	V
Oligohydramnios/polyhydramnios	+	+	+	+	+
Donor bladder is no longer visible	–	+	±	±	±
Hemodynamic disturbance (AREDF in the umbilical artery, umbilical venous pulsatility, absent flow or negative a-wave in ductus venosus)	–	–	+	+	+
Generalized edema in at least one fetus	–	–	–	+	+
Intrauterine fetal demise of at least one fetus	–	–	–	–	+

AREDF — absent or reversed end-diastolic flow

anastomoses between the fetuses: more blood flows through the vascular anastomoses from the 'donor' twin (donor) to the 'recipient' twin (recipient) [147]. The prevalence of TTTS has been estimated at 5–15% of all monochorionic twin pregnancies [148, 149].

Diagnosis of TTTS

Oligohydramnios in the donor and polyhydramnios in the recipient, with a shared chorion for at least two fetuses, is the basis for the diagnosis of TTTS, if the following conditions are met:

— oligohydramnios: MVP or deepest vertical pocket (DVP) of ≤ 2 cm;
— polyhydramnios: MVP of ≥ 8 cm regardless of GA [147] or ≥ 8 cm until 20 weeks GA, ≥ 10 cm afterwards [150].

The Quintero Staging System — which is used to evaluate the severity of TTTS — is presented in Table 9 [151].

Fundamentals of TTTS management:

— isolated TTTS is not an indication for invasive diagnostics to test for genetic abnormalities;
— patient should be referred to high-level of care center for antenatal therapy;
— elective cesarean section is the recommended mode of delivery for TTTS with expectant management [152];
— TTTS is associated with elevated risk for ischemic and thrombotic complications for the twins (especially the recipient), which might result in deformity or limb deficiency — that complication has also been reported in pregnancies without laser therapy [153–155].

Fetal therapy

Fetoscopic laser ablation of the placental anastomoses remains the standard of care for TTTS. The diagnosis poses little, if any, challenge for an experienced sonographer and is the main eligibility criterion for intrauterine therapy. Still, the eligibility determination process may be contestable in ambiguous cases, especially in stage I TTTS (Quintero) with no clinical manifestations (polyhydramnios, short cervix) and in fetuses at < 16 and > 26 weeks GA [156–159].

Table 10. Management in twin-to-twin transfusion syndrome (TTTS) versus gestational age

< 16 weeks	16–26 weeks	> 26 weeks
Expectant management	Fetoscopy	Amnioreduction Steroid therapy Neuroprotection delivery

Exclusion criteria for laser therapy: premature rupture of membranes, uterine contractility, coagulation disorders, technical obstacles, blood-stained amniotic fluid (relative contraindication), chorioamniotic separation or septostomy after amniocentesis or amnioreduction (relative contraindication).

Risk factors: proximate cord insertion, chorioamniotic separation, GA < 16 and > 26 weeks [158, 160, 161]

Types of intrauterine procedure

The recommended management — depending on gestational age — is presented in Table 10.

Benefits of intrauterine intervention [149, 156, 162, 163]

Technically successful laser ablation of the fetal anastomoses improves survival and neonatal outcomes but does not guarantee that both fetuses will be saved. The survival rates and risk for CNS damage for different types of management are presented in Table 11.

Complications after the procedure

The most common complications after laser therapy in TTTS include: rupture of the membranes, vaginal bleeding or into the abdominal cavity, uterine contractility, intrauterine infection, pulmonary edema, amniotic fluid embolism, and amniotic fluid leakage into the maternal peritoneal cavity [164, 165].

Post-procedure management

1. Weekly follow-up for the first 2 weeks postoperatively — afterwards at the discretion of the physician (every

Table 11. Survival rate and risk for central nervous system (CNS) damage versus choice of treatment

	Expectant management	Amnioreduction	Fetoscopy
Survival of at least one fetus	< 10%	30–83%	76–90%
Survival of both fetuses	< 10%	20–80%	36–70%
CNS damage	50%	14%	6%

1–2 weeks): biometric parameters; MVP; blood flow in the umbilical vessels, middle cerebral artery, ductus venosus; evaluation of the brain, heart, and limbs.

2. If one twin died after the procedure: neurosonography or MRI 4–6 weeks after the intervention [166].

Recommended mode of delivery after intrauterine intervention

Fetoscopic procedure is not an absolute indication for a cesarean section. If ultrasound manifestations of TTTS or twin anemia-polycythemia sequence (TAPS) persist, delivery after 34 weeks GA should be considered [149, 166]. The final decision about the mode of delivery remains at the discretion of an experienced obstetric team.

Selective fetal growth restriction (sFGR) in monochorionic twin pregnancy

Selective fetal growth restriction is characterized by significantly restricted growth of one fetus. Selective fetal growth restriction is believed to be caused by unequal sharing of the placenta and the resulting insufficient transfer of oxygen to the smaller fetus. The prevalence of sFGR has been estimated at 10–15% of all monochorionic twin gestations [167].

Diagnosis

The so-called 'Delphi definition' for sFGR is used in the diagnostic process: detection of one solitary parameter or at least two out of four contributory parameters (Tab. 12) [168].

According to the Fetal Medicine Foundation guidelines, all three of the following criteria need to be met for the sFGR to be diagnosed:

— EFW < 5th centile;
— EFW discordance between the fetuses of ≥ 25%;
— decreased AFV in the smaller twin but normal AFI in the other twin [169].

Selective fetal growth restriction severity

According to the criteria published by Gratacós et al. [170], the umbilical artery Doppler flow in the smaller twin may be used to assess the severity of sFGR :

— type I: normal umbilical artery (UA) Doppler;
— type II: absent or reversed end-diastolic flow in the UA;
— type III: intermittent absent-reversed end-diastolic flow in the UA.

Table 12. Diagnostic criteria for selective fetal growth restriction (sFGR)

Hypotrophic features	Monochorionic pregnancy
Solitary	EFW of 1 of the fetuses < 3rd centile
Contributory	EFW of 1 of the fetuses < 10th centile
	AC of 1 of the fetuses < 10th centile
	EFW discordance ≥ 25%
	PI in the umbilical artery of the smaller fetus > 95th centile

EFW — estimated fetal weight; AC — abdominal circumference; PI — pulsatily index

Fetal therapy

The diagnosis of sFGR with high risk for intrauterine fetal demise (type II but also type III, according to some authors) — is the main eligibility criterion for laser ablation of fetal anastomoses and separation of the fetal venous circulations [171]. In some centers, umbilical cord occlusion of the hypoxic twin is recommended. The procedure is performed to minimize the risk for intrauterine fetal demise of the eutrophic twin. The intervention is contraindicated in the following cases: PROM, uterine contractility, coagulation disorders, technical obstacles (little chance for a successful procedure), blood-stained amniotic fluid after amniocentesis or amnioreduction (relative contraindication).

Higher risk for complications is associated with the following parameters

— proximal cord insertion;
— chorioamniotic separation;
— GA < 16 and > 26 weeks.

Results of the intrauterine intervention are presented in Table 13.

Intrauterine interventions have negligible effect on the prognosis: the mortality rate after the procedure is similar to that observed in expectant management, or higher according to some sources (cord occlusion, post-procedure complications), and the prevalence of damage to the CNS is comparable, slightly less frequent in the normal-weight

Table 13. Results of intrauterine management of selective fetal growth restriction (sFGR)

	Expectant management	Cord occlusion	Ablation of placental anastomoses
Intrauterine fetal demise	4.6–32.7%	53.4–58%	44.3–46.8%

twin and more frequent in the hypotrophic twin. According to most sources, the prognosis for the larger twin improves after cord occlusion in the hypotrophic twin.

Complications after the procedure

The most common complications after laser ablation for sFGR include rupture of the membranes, bleeding from the genital tract or into the abdominal cavity, uterine contractility, intrauterine infection, amniotic fluid leakage into the maternal peritoneum [164, 167].

Post-procedure management

1. Weekly follow-up for the first 2 weeks postoperatively — afterwards at the discretion of the physician (every 1–2 weeks): biometric parameters, MVP, blood flow in the umbilical vessels, middle cerebral artery, ductus venosus, evaluation of the brain, heart, and limbs.
2. If one twin died after the procedure: neurosonography 4–6 weeks after the intervention [165].

Delivery

Fetoscopic intervention is not an absolute indication for a cesarean section. Nevertheless, the mode of delivery depends on the number of live fetuses, their presentation, EFW, potential threat to fetal wellbeing, and fetal hypoxia. If type I hypotrophy is found in the second twin, and in the absence of hypoxia in that twin, vaginal delivery remains an option for pregnancies which do not require laser ablation. Hypotrophy in the first twin, non-cephalic presentation of the first twin and symptoms of fetal hypoxia are indications for a cesarean delivery. The final decision should remain at the discretion of the obstetric team.

Twin reversed arterial perfusion (TRAP)

Twin reversed arterial perfusion develops in a monochorionic pregnancy as a result of abnormal arterioarterial anastomoses in the placenta, with all the blood flowing directly from one fetus to the other. Reversed blood flow in the aorta and a single umbilical artery are typically observed. The body structures of the recipient located above the chest cavity (mainly the head and the upper extremities) will atrophy in the initial stages of the embryonic development due to lack of normal perfusion and tissue nutrition. As the blood flowing through the arterioarterial anastomoses bypasses the placental circulation, it is deoxygenated and lacks nutrients, but it is rich in metabolites of the donor twin.

The donor twin is also known as the 'pump twin,' while the recipient is also known as the 'acardiac' or 'parasitic' twin. As far as the latter twin is concerned, although the term 'acardiac' is more commonly found in the literature, it is not entirely correct as sporadic heart activity may be observed in the theoretically acardiac fetus. The term 'parasitic' is more accurate from the pathophysiological point of view. It is also useful during counselling, when umbilical cord laser ablation of the 'parasitic' fetus is advised. From the psychological as well as medical and legal point of view, this procedure is dissimilar to embryo reduction.

Twin reversed arterial perfusion may only develop in monochorionic pregnancies, monoamniotic as well as diamniotic. At present, the prevalence of TRAP is estimated at 2.6% of monochorionic pregnancies, *i.e.* from 1:9500 to 1:11000 of all gestations, depending on the number of pregnancies achieved using ART and kinds of techniques used in a given population [172].

Approximately 50% of the pump twins die due to congestive heart failure or extreme prematurity due to rapidly progressing polyhydramnios. TRAP has been also reported in a triplet monochorionic pregnancy or even a quadruplet pregnancy.

Diagnosis

The diagnosis of TRAP is typically made between 11–14 weeks GA. The parasitic twin is severely malformed, in most cases the head and the upper limbs are not developed. The lower limbs are developed and mobile. Blood flow in the fetal aorta and the umbilical artery is reversed. Sporadically, at the initial stages of pregnancy, the fetal heart, residual cranial structures, and even upper limbs may be identified.

Indications for invasive diagnostics

The literature offers reports about an elevated risk for chromosomal aberrations in the TRAP syndrome. During the procedure, amniotic fluid is routinely sampled for fetal karyotyping or chromosomal microarray analysis (aCGH).

Fetal therapy

Several methods of vessel occlusion to stop the blood flow to the parasitic twin have been described but their value nowadays is mostly historic. At present, the microinvasive laser coagulation of the intraabdominal vessels of the parasitic twin is the method of choice for TRAP, in cases with timely diagnosis. At late diagnosis, *i.e.*, when the

abovementioned method would be unsuccessful, laser occlusion of the umbilical vessels of the parasitic twin remains an option. Approximately 45% of the pump twins survive the expectant management. Postoperative survival rate is 80% for interventions performed after 16 weeks GA. Delayed intervention until 16–18 weeks GA is associated with 60% risk for spontaneous fetal demise in the acardiac twin, and with hemorrhaging to the CNS or fetal death of the pump twin in 60% of the cases. It is recommended to schedule the procedure immediately after the diagnosis of TRAP is confirmed [173].

Eligibility determination process

— TRAP;
— GA at intervention: 12–14 weeks;
— at late presentation or diagnosis (> 23 weeks GA) — individual eligibility process at a high-level care center is advised [173, 174].

Exclusion criteria

late diagnosis, advanced gestational age with low-intensity hemodynamic changes and polyhydramnios.

Types of intrauterine interventions

Microinvasive laser coagulation of the intraabdominal vessels of the parasitic twin involves an ultrasound-guided introduction of a 18G-needle and 400 µm in outer diameter optical fiber (or 17G needle and 600 µm optical fiber) into the parasitic twin and coagulation of the umbilical artery in the pelvis, the iliac arteries, and distal parts of the aorta [175].

At late diagnosis — depending on the clinical situation and experience of the center — fetoscopic laser occlusion of the parasitic twin umbilical cord vessels or bipolar diathermy coagulation may be used. In a monochorionic monoamniotic gestation, it is prudent to consider cord resection of the parasitic twin to avoid cord entanglement later on, which might lead to the demise of the pump twin [176, 177].

A significantly improved chance for the birth of a healthy child is the main benefit of the intrauterine intervention. Complications include ineffective coagulation of the blood vessels (recurrent perfusion), risk for neurologic complications, and death of the pump twin due to hemorrhage.

Monitoring

If the intrauterine intervention proved to be effective, ultrasound monitoring of the remaining fetus is recommended on postoperative days: 2, 7, and 14, followed by a check-up visit every two weeks. During the first and second postoperative ultrasound, particular attention should be paid to developmental abnormalities in the CNS of the healthy fetus. In case of late diagnosis/expectant management, the frequency of check-up visits should be individually assigned to each patient at the fetal therapy center.

Delivery

The mode of delivery depends on the obstetric status. If the intrauterine intervention was successful, there are no indications for a cesarean section.

Twin anemia-polycythemia sequence

Twin anemia-polycythemia sequence is a rare complication of a twin or multifetal monochorionic pregnancy. It is a form of acute feto-fetal hemorrhage (described in 2007 by Lopriore et al.) resulting from blood flow from one fetus (donor) to the other (recipient) through extremely small arteriovenous anastomoses (< 1 mm in diameter). The absence of the polyhydramnios-oligohydramnios sequence differentiates TAPS from TTTS [178, 179].

Twin anemia-polycythemia sequence may develop spontaneously or as a complication after laser photocoagulation of the fetal anastomoses for TTTS. Due to low prevalence, the findings of statistical analyses for TAPS remain disputable and estimative. The prevalence ranges from 1.6% to 5% of all monochorionic diamniotic gestations for spontaneous TAPS and 16% after fetoscopic laser therapy for TTTS. It is important to differentiate between TAPS and Acute Feto-Fetal Hemorrhage (AFFH), which may develop after labor.

Diagnosis

Antenatal diagnosis of TAPS is based on the measurements of the middle cerebral artery peak systolic velocity (MCA-PSV): > 1.5 MoM in the donor and < 1.0 MoM in the recipient. Postnatal diagnostics involves detection of a significant intertwin difference in hemoglobin (Hb) concentration in the neonatal blood (> 8 g/dL), and one of the two symptoms: reticulocyte index of > 1.7 or the presence of small anastomoses on the surface of the placenta (Fig. 8). Prolonged erythroblastosis in the donor, which is indicative of chronic anemia, has also been described [180]. Stages of TAPS are presented in Table 14.

Fetal therapy

Causative management

The causative management uses laser photocoagulation of the anastomoses, like in case of TTTS. The absence of polyhydramnios in one of the amniotic sacks, lower amniotic fluid clarity and non-smooth surface of the fetal placenta impede identification of the anastomoses, which are small and often peripheral, making the procedure moderately challenging. Still, the method grows in popularity because it is a causative management, but also because longer duration of pregnancy was achieved in patients undergoing laser coagulation of the anastomoses,

even if it is associated with an elevated risk for PPROM. Postnatally, intertwin hemoglobin difference is less pronounced, the Hb levels return to the normal values more swiftly, and the discrepancy in fetal weight is less significant, mainly as a result of improved intrauterine growth in the donor [181].

Symptomatic management

Intrauterine transfusion to the anemic donor is used as a form of symptomatic treatment in cases when technical obstacles impede the laser intervention. Despite considerable experience of the operators in intrauterine transfusions directly to the umbilical vein, some authors suggest using the intraperitoneal transfusion which — by slowing down the absorption of the red blood cells — is supposed to prevent their immediate transfer to the circulation of the recipient twin. That technique is considered to be a temporary solution. Also, it has been suggested to conjoin intrauterine fetal transfusion in the donor with partial exchange transfusion in the recipient to lower its polycythemia. In selected cases, blood obtained from the recipient twin may be transferred to the donor twin instead of blood from another donor. The method is not without limitations, chief among them the need for a double cordocentesis, both in the donor and the recipient, increasing the intervention-related risk. If possible, it is advised to secure allogeneic Rh-matched RBC concentrate, same as for intrauterine transfusions, as it is safer for the mother.

Despite the still existing anastomoses, TAPS recurrence rate after the transfusions is low and the need for repeat transfusions decreases after blood transfusions. Also, the mechanism of anastomotic thrombosis, disabling the existing vascular connections, has also been suggested [181].

Figure 8. Small anastomoses on the surface of the placenta

Management of iatrogenic TAPS

In case of iatrogenic TAPS — after laser therapy for TTTS — yet another procedure is not always effective and if the small anastomoses had not been identified during the first surgery, it might be challenging to identify them during the subsequent intervention. The Solomon technique is recommended during the first laser surgery for TTTS to avoid such cases [181].

Eligibility process

In light of the fact that the survival rates are similar for expectant as well as active management (94% — laser therapy; 84% — expectant management and transfusion), expectant management is advised, while active management is recommended only in severe TAPS. In such cases, higher prevalence of cardiomyopathy and hypertension have been reported in the recipients. Also, elevated creatinine levels in the donors are indicative of transient renal dysfunction [182].

Data on damage to the central nervous system in fetuses with TAPS remain conflicting — intellectual disability and spastic paraplegia have been reported. Inconsistent observational study samples as far as the causal factor for TAPS is concerned, especially cases after earlier laser therapy for TTTS, are believed to be responsible for those inconsistencies. Small sample size of the studies has also been mentioned. Nevertheless, neurological deficits have been observed both, in donors and recipients, although less often in spontaneous TAPS [181, 183–185].

Table 14. Twin anemia-polycythemia sequence (TAPS) stages

Stage	Antenatal	Postnatal difference in Hb concentration
1	Donor MCA-PSV > 1.5 MoM recipient MCA-PSV < 1.0 MoM	> 8 g/dL
2	Donor MCA-PSV > 1.7 MoM recipient MCA-PSV < 0.8 MoM	> 11 g/dL
3	As in stage 1 or 2 + critical cardiac compromise	> 14 g/dL
4	Hydrops in the donor	> 17 g/dL
5	Fetal demise of one or both twins preceded by TAPS	> 20 g/dL

MCA-PSV — middle cerebral artery peak systolic velocity; MoM — multiple of median

Complications

It is necessary to be vigilant about possible complications in pregnant women with TAPS who present with pulmonary embolism or the mirror syndrome [186]. Due to the severity of the pathology and variety of therapies, care over patients with TAPS requires considerable experience and should be offered at a tertiary referral center. In the absence of definite guidelines, the therapy needs to be tailored to the individual needs of the patient, depending on the experience of the perinatologist team and the clinical situation.

Monitoring and check-up visits

Check-up visits at least every 2 weeks with normal fetal parameters, and at least once a week when signs of deteriorating fetal wellbeing appear, are necessary for timely detection of pathological findings in a monochorionic twin pregnancy [186, 187].

Delivery

The literature offers guidelines on the mode and timing of delivery for uncomplicated monochorionic pregnancies but lacks clear recommendations for gestations complicated with TAPS. Therefore, it is safe to assume that vaginal delivery, as per the monochorionic delivery protocol, is possible if the obstetric team can monitor for acute feto-fetal hemorrhage during labor. The decision about timing should remain at the discretion of an experienced team of perinatologists [186, 187].

Article information and declarations

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Conflict of interest

All authors declare no conflict of interest.

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Three natural pregnancies following embolization of both uterine arteries due to pseudoaneurysms associated with the gestational trophoblastic disease — long-term follow-up

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INTRODUCTION

Uterine artery pseudoaneurysm (UAP) is an abnormal single-layer outpouching of the arterial wall [1]. Its reported frequency rate is 0.2% [2]. UAP mainly forms following invasive uterine procedures, like cesarean section, and presents as vaginal bleeding [1]. Doppler ultrasonography is the universal early diagnostic tool, but computer tomography angiography remains a gold standard. Nevertheless, magnetic resonance imaging is a radiation-free alternative. Treatment options include arterial embolization and hysterectomy, depending on the clinical status. Endovascular occlusion is considered a method of choice, especially in patients wishing to preserve fertility [1].

CASE REPORT

A twenty-six years old female gravida 1, para 0, with a history of successful gestational trophoblastic disease treatment, was admitted in March 2009 due to asymptomatic pseudoaneurysms. In February 2009, a UAP embolization attempt in another institution resulted in the left uterine artery occlusion, with no decrease in pseudoaneurysms sizes.

Upon admission, the Duplex ultrasound and MRI confirmed the diagnosis of pseudoaneurysms. Digital subtraction angiography revealed the right uterine artery supplying three UAPs in the uterine corpus measuring: 50 × 45 mm, 12 mm, and 8 mm, which were embolized using glue (N-butyl-2-cyanoacrylate). Final arteriography confirmed successful embolization with the closure of most right uterine artery branches. Aortography visualized ovarian arteries as the only remaining arterial supply to the area, both enlarged to 3 mm. Post-procedure ultrasound and MRI showed a mass of the pseudoaneurysms with no perfusion. A follow-up MRI 12 months post-treatment showed a small fibrotic lesion (17 mm) in the place of previous pseudoaneurysms. Following the embolization, the patient achieved three healthy pregnancies (2010, 2012, and 2017). The infants were delivered vaginally in physiological time, with birth weights of 2800 g, 3150 g, and 3200 g, respectively. As of June 2023, the patient had no procedure-related complications. All children are meeting the expected developmental milestones.

DISCUSSION

Embolization of the uterine arteries is a standard treatment method for extravaginal bleeding that allows for precise determination of the bleeding site. Moreover, it shows lower morbidity compared to surgery and has a potential for fertility

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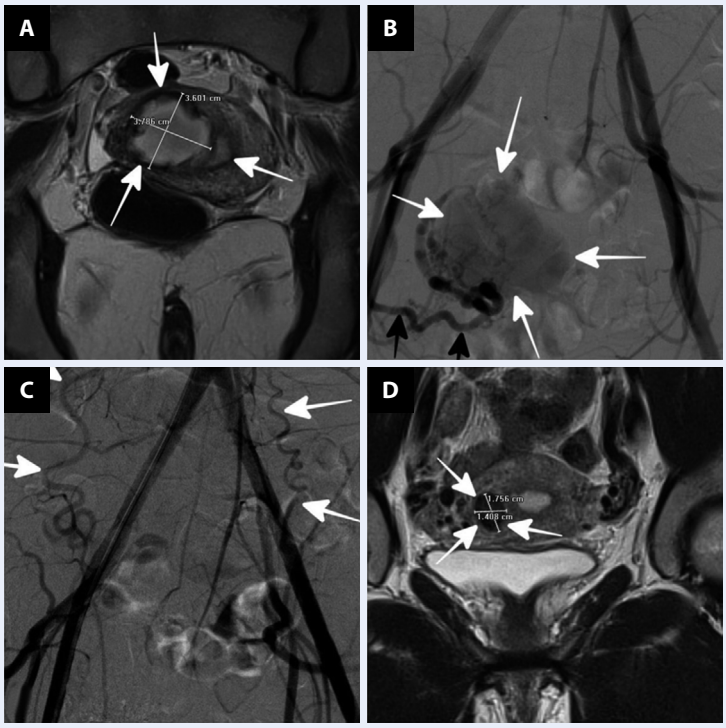

Figure 1. A. Coronal magnetic resonance imagining (MRI) with visible uterine aneurysm (arrows)**; B.** Digital subtraction angiography of the pelvis before embolization. Contrast agent in the iliac and right uterine arteries (black arrows) and uterine aneurysm (white arrows); **C.** Digital subtraction angiography of the pelvis after embolization. Invisible uterine arteries and aneurysms. Arrows indicate bilaterally dilated ovarian arteries; **D.** Sagittal MRI of the pelvis 12 months after embolization. Arrows indicate fibrotic uterine aneurysm

preservation [3]. Available research suggests bilateral internal iliac artery ligation is more effective than unilateral ligation in controlling extravaginal bleeding [4], perhaps due to contralateral neovascularization [5]. However, in the case of non-bleeding pseudoaneurysms, a selective closure of uterine arteries seems more advantageous. The decision of glue use was based on the high number of pseudoaneurysms and multiple inflow arterial branches. Post-procedurally, the patient's uterus and ovaries were perfused solely by the ovarian arteries, which supplied sufficient blood for the proper development of three normal pregnancies. This suggests that the compensation capability of the pelvic arterial system is underestimated in daily routine practice.

CONCLUSIONS

Selective embolization of the uterine arteries can be a successful treatment method for UAP, providing efficient hemostasis and preserving uterine function. The ovarian arteries alone are sufficient to supply the uterus during pregnancy without adversely impacting child development.

Article information and declarations

Ethics statement

It was a retrospective clinical vignette, which is why no bioethics committee approval was required. The patient agreed to publish the outcomes of her treatment.

Author contributions

Radoslaw Pietura — concept, assumptions, aquisition of data, analysis and interpretation of data, critical revision of the article.
Slawomir Wozniak — concept, assumptions, critical revision of the article.
Michal Toborek — assumptions, aquisition of data, analysis and interpretation of data, article draft.
Kinga Kwolek — analysis and interpretation of data, article draft and editing.
Natalia Pietron — analysis and interpretation of data, article draft and editing.

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Conflict of interest

The authors declare no conflict of interest.

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Prenatal diagnosis of isolated total anomalous pulmonary venous connection (TAPVC) to coronary sinus

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Total anomalous pulmonary venous connection (TAPVC) is the fifth most common cyanotic heart disease, affecting 5.9–7.1 per 100,000 live births [1, 2].

The main feature is that all four pulmonary veins (PV) are not connected to the left atrium (LA) but to the systemic veins, coronary sinus (CS), or right atrium (RA). Four TAPVC types are distinguished based on the PV connect sites: supracardiac, cardiac, infracardiac, and a mixed type. TAPVC can occur as part of complex cardiac malformations, especially in heterotaxy syndrome, or in isolation. Prenatal assessment of the fetal venous system and left venoatrial junction is part of the four-chamber-view evaluation, as stipulated in national and international fetal cardiac screening guidelines [3, 4], however assessment is challenging, and reports indicate that only 1.9% of TAPVC cases are diagnosed prenatally [5].

Prenatal diagnosis of TAPVC is crucial, as it explains postnatal cyanotic heart defect symptoms and enables prompt, sometime lifesaving, treatment planning. The presence of an obstruction at any level of the venous route is the most critical factor affecting outcome and management of the defect, making the defect one of the few true neonatal cardiac emergencies. Prognosis is very good if detected prenatally and corrected during the neonatal period [2].

The study aim was to identify characteristic sonographic features of TAPVC where there was PV drainage into the CS.

A 35-year-old primigravida at 26 weeks of pregnancy was referred to our department because PV identification by imaging was impossible. Examination revealed normal visceral situs, normal heart size, drainage of the systemic veins into the RA, and concordant atrioventricular connection. The space between the spine and the LA drew our attention as the four PV drained through a collector to the enlarged CS and then to the RA (Fig. 1A, Suppl. video). Imaging also identified the enlarged width of the CS (Fig.1B). In colour and PW Doppler modes, the blood flow spectrum was normal (Fig. 1C). The outflow tracts and upper mediastinum were normal. During the third trimester, the RA and RV became enlarged, and the LA and LV volumes were smaller than normal, however the LV depth was normal and formed the apex of the heart. Trivial tricuspid regurgitation was also detected. Coronary sinus width was 4.9 mm. With TAPVC suspected, examination by a pediatric cardiologist confirmed the diagnosis. The neonate was born at 39 weeks of gestation with vitals of body weight 3300 g, Apgar score 10, venous blood pH = 7.321, and $satO_2$ = 90%.

Postnatal echocardiography and cardiac angiography confirmed that all PV formed a collector behind the LA, and the collector drained into the CS with its kinking (Fig. 1D, Fig. S1). At the level of the CS kinking, a flow gradient of max 16 and mean 12 mm Hg was revealed. The right atrium and ventricle were significantly enlarged (Fig. 1E, Fig. S2), with severe hypertrophy of the right ventricle free wall but normal contractility. Trivial tricuspid valve insufficiency with a retrograde systolic maximal gradient of 34–43 mm Hg, normal right ventricle outflow tract, and widened right and left pulmonary arteries (RPA = 5.2 mm, LPA = 4.7 mm) were observed. Pulmonary blood flow with hypertension symptoms was diagnosed. Atrial septal defect type II of 6 mm with right to left shunt was detected. Left ventricle output was 2.2 L/min/m^2, with normal ejection 75% and shortening fraction 40%. The mitral valve was competent, and the aortic

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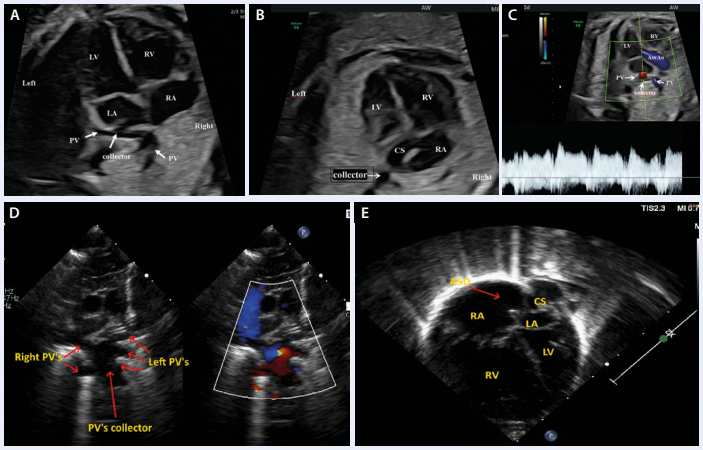

Figure 1. A. Four chamber view at 26 weeks of gestation; the pulmonary veins join a collector visible behind the posterior wall of the left atrium; **B.** Enlarged coronary sinus at 26 weeks of gestation; **C.** 26 weeks of gestation; colour Doppler mode; unrestricted blood flow through the collector; **D.** Postnatal suprasternal view: right and left pulmonary veins draining to pulmonary vein collector. See arrows; **E.** Postnatal modified 4-chamber view: enlarged right atrium (RA) and right ventricle (RV), small left atrium (LA), compressed left ventricle (LV), CS indicates the point where pulmonary collector connects to coronary sinus, the red arrow shows atrial septal defect position (ASD); Ao — aorta; CS — coronary sinus; PV — pulmonary vein

valve was tricomissural and trileaflet, and functioning normally. The coronary arteries originated normally. Imaging identified a normal left aortic arch with normal wide isthmus.

Total correction was done on the 10th day of life: CS drainage was converted from the right to the left atrium. The post-surgery course was not complicated. Follow-up examination proved non-restrictive blood flow through the established coronary sinus to the LA connection. The ventricular systolic function and heart output were normal. The neonate was discharged on the 15th day of life in good general condition, with arterial SatO2 92-95%. Out-patient follow up indicated the child was healthy and developing well.

In conclusion, though difficult, TAPVC can be diagnosed using antenatal ultrasound. In our case, 2D and colour Doppler were chosen for diagnosis. In-utero diagnosis enabled a multidisciplinary approach and optimal prenatal and postnatal patient counselling and treatment.

Article information and declarations

Ethics statement

The institutional review board waived the requirement for a separate ethical approval for this clinical vignette, since the sonographic evaluations were performed as integral parts of routine clinical care, for which informed consent had been previously given by the patient. Data were anonymized.

Author contributions

Anna Wojtowicz — conecpt,analysis and interpretation of data, article draft, literature review, corresponding author, Beata Zaluska-Pitak — collecting data, article draft, literature review, Mgadalena Juszcak — literature review, Hubert Huras — literature review, Sebastian Goreczny — verification, literature review.

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None.

Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary video and Figures S1–S2 available on https://journals.viamedica.pl/ginekologia_polska/article/view/gpl.96430

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