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Evaluation and Management of Primary Amenorrhea

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Abstract

Primary amenorrhea is the absence of menarche in ≥15-year-old females with developed secondary sexual characteristics and normal growth or in ≥13-year-old females without signs of pubertal development. It is a diagnostic challenge due to its diverse etiologies.

We begin by presenting a case of a 17-year-old girl referred for primary amenorrhea, with normal pubertal development and unremarkable physical examination. Initial blood tests lead to the hypothesis of hypogonadotropic hypogonadism. However, subsequent blood tests after cessation of oral contraceptives showed normal results, while pelvic ultrasound revealed a poorly individualized uterus. Ultimately, she was diagnosed with Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome, characterized by congenital absence of the uterus.

Through a comprehensive review, we outline the spectrum of causes of primary amenorrhea, including anatomic defects, primary hypogonadism, hypothalamic-pituitary disorders, and other endocrine abnormalities. Diagnostic evaluation involves a detailed medical history, physical examination, and targeted laboratory and imaging studies to elucidate the underlying pathology.

This work highlights the importance of a systematic approach to primary amenorrhea to ensure timely diagnosis and appropriate management.

KEYWORDS: Primary amenorrhea, Hypogonadism, Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome

Resumo

A amenorreia primária é definida pela ausência de menarca em meninas de ≥15 anos com caracteres sexuais secundários desenvolvidos e crescimento normal ou em meninas de ≥13 anos sem sinais de desenvolvimento pubertário.

Apresentamos o caso de uma adolescente de 17 anos encaminhada por amenorreia primária, com desenvolvimento pubertário normal e exame físico sem alterações. Os exames de sangue iniciais levaram à hipótese diagnóstica de hipogonadismo hipogonadotrófico. No entanto, exames subsequentes após a interrupção dos contraceptivos orais mostraram resultados normais. A ultrassonografia pélvica revelou um útero pouco individualizado. O diagnóstico final foi de Síndrome de Mayer-Rokitansky-Kuster-Hauser, caracterizado por ausência congénita do útero.

Após a exposição do caso, através de uma revisão da literatura, são apresentadas as causas de amenorreia primária incluindo defeitos anatómicos, hipogonadismo primário, distúrbios hipotalâmico-hipofisários e outras patologias endócrinas. A avaliação diagnóstica deve ser baseada numa história médica detalhada e exame físico completo, suportados pela avaliação laboratorial e de imagem direcionados.

Com este trabalho, pretende-se realçar a importância de uma abordagem sistemática da amenorreia primária, de modo a garantir um diagnóstico célere e uma abordagem adequada.

PALAVRAS-CHAVE: Amenorreia Primária, Hipogonadismo, Síndrome de Mayer-Rokitansky-Kuster-Hauser (MRKH)

Index

1. Abbreviations

17-OHP - 17-hydroxyprogesterone

AG- Adrenal gland

AIS - Androgen insensitivity syndrome

CDGP- Constitutional Delay of Growth and Puberty

CHH - Congenital Hypogonadotropic Hypogonadism

CT - Computed tomography

DHEA - Dehydroepiandrosterone

DHEA -S - Dehydroepiandrosterone Sulfate

DHT - Dihydrotestosterone

FIGLA - Factor in Germline alpha

FSH - Follicle Stimulating hormone

FSHβ - FSH beta subunit

FT4 - Free thyroxine

GH – Growth hormone

GnRH - Gonadotropin-releasing hormone

HPO - Hypothalamus - Pituitary - Ovarian

IGF-1 - Insulin-like growth factor 1

LH - Luteinizing hormone

LHRH - Luteinizing hormone-releasing hormone

LHβ - LH beta subunit

MRI- Magnetic resonance imaging

MRKH - Mayer-Rokitansky-Kuster-Hauser

NCAH - Non-Classic Congenital Adrenal Hyperplasia

NOBOX - Newborn Ovary Homeobox

NR5A1 - Nuclear receptor subfamily five group A member 1

PCOS- Polycystic Ovary Syndrome

POI - Primary Ovarian Insufficiency

PRL – Prolactin

PROP1 - PIT1 prophet gene

RV – Reference Value

2. Methods

To write this work, we consulted the patient's medical chart and considered the clinical history and physical examination records.

Subsequently, a clinical discussion was conducted based on a literature review. Literature was selected from a structured search on the PubMed® platform between December 2023 and February 2024. The terms searched were: Primary Amenorrhea and Primary Amenorrhoea. Only articles written in English and fully available were considered. Afterwards, articles were selected and read based on the title and abstract, and the most relevant information was collected according to the purpose of this work.

3. Introduction

Menstruation is an indicator of general well-being and health. Therefore, irregular menstrual patterns or absence of menses can be alarming as they may indicate poor health. (D. A. Klein et al., 2019)

Amenorrhea is the absence of menstruation and can be classified into primary or secondary. Primary amenorrhea refers to the absence of menarche, the first menstruation, in ≥15-year-old females with developed secondary sexual characteristics and normal growth or in ≥13-year-old females without signs of pubertal development. (Seppä et al., 2021) Secondary amenorrhea consists in the lack of menstruation for three or more months if previous menstrual cycles were regular, or six or more months if they were irregular. (D. A. Klein et al., 2019)

Many conditions, including gynecological abnormalities, pituitary disorders, and chronic diseases can present as amenorrhea. (D. A. Klein et al., 2019) For this reason, a wide investigation is important to avoid missing significant diagnoses.

According to the American Society for Reproductive Medicine, the causes of amenorrhea can be divided into six main categories: anatomic defects (outflow tract defects), primary hypogonadism, hypothalamic causes, pituitary causes, other endocrine gland disorders, and multifactorial causes. (‘Current Evaluation of Amenorrhea’, 2008)

Regarding secondary amenorrhea, the main etiologies are polycystic ovary syndrome, hypothalamic amenorrhea, hyperprolactinemia, and primary ovarian insufficiency. (D. A. Klein & Poth, 2013)

I will report a case of primary amenorrhea and review the literature on the etiology and management of primary amenorrhea.

4. Case Report

A 17-year-old girl was referred to the endocrinology outpatient clinic for primary amenorrhea.

Apart from a head trauma when she was four months of age, her personal history was irrelevant. Her family background also held no significance.

She reported her thelarche at the age of 11, and her puberty had progressed normally during the following years. She also had adequate psychomotor and cognitive development. At the age of 14, although menarche had still not occurred, she started an oral contraceptive due to acne and self-reported hirsutism. Even under oral contraceptive, there was no uterine bleeding. She denied any cyclical pain or discomfort, as well as galactorrhea, visual disturbances, headache, anosmia, or hyposmia.

On her first pediatric endocrinology appointment, at the age of 17, her physical exam was unremarkable. Her weight was 50,8 Kg and her height was 155,1 cm (normal according to the target height based on midparental height). She had complete pubertal development and no dysmorphias, no signs of hirsutism or virilization.

She carried out some blood tests, shortly after stopping the oral contraceptive, that revealed normal-low gonadotropins (Table 1). Thyroid function and androgens, including total testosterone, were normal. The laboratory evaluation revealed no other abnormality and the karyotype was 46, XX.

Table 1- Patient's results at the initial evaluation

Parameter	Patient's results	Reference Value (RV)
Follicle Stimulating hormone (FSH) (U/L)	5,8	3,5 – 12,5 follicular phase
Luteinizing hormone (LH) (U/L)	6,0	2,4 – 12,6 follicular phase
Dehydroepiandrosterone Sulfate (DHEA-S) (ug/dL)	360	65,1 - 368
Total Testosterone (ng/dL)	46	5-48
Thyroid Stimulating hormone (TSH) (uU/mL)	1,45	0,51-4,3
Free thyroxine (FT4) (ng/dL)	1,26	0,9-1,63
17-hydroxyprogesterone (OH) (ng/mL)	0,6	0,1-0,8
Prolactin (PRL) (ng/mL)	8,6	4,7-23
Insulin-like growth factor 1 (IGF-1) (ng/mL)	379	161-479

At this time, it was hypothesized that the patient might have an isolated hypogonadotropic hypogonadism. Although she had reported normal pubertal development, there were no medical records that could confirm this. Also, it seemed possible that her normal development was due to exposure to estrogens in the oral contraceptives she had been taking since the age of 14. For this reason, she underwent a luteinizing hormone-releasing hormone (LHRH) stimulation test, three months after stopping the oral contraceptive, and a pituitary magnetic resonance imaging (MRI).

The simulation test showed an appropriate rise in gonadotropins and estradiol (Table 2), excluding hypogonadotropic hypogonadism. Her MRI was also normal.

Table 2- Patient's results on the LHRH stimulation test.

Parameter	Patient's results	Reference Value (RV)
Estradiol (pg/mL)	153	12-233 follicular phase
FSH (U/L) – at minute 0	1,7	3,5 – 12,5 follicular phase
LH (U/L) – at minute 0	2,2	2,4 – 12,6 follicular phase
FSH (U/L) – at minute 30	3,59	-
LH (U/L) – at minute 30	36,3	-
FSH (U/L) – at minute 60	4,5	-
LH (U/L) – at minute 60	35,4	-
FSH (U/L) – at minute 90	5,14	-
LH (U/L) – at minute 90	36,1	-
FSH (U/L) – at minute 120	5,8	-
LH (U/L) – at minute 120	41,6	-

Normal response: LH rises at 30-60 minutes 6-10 times basal; FSH rises at 30-60 minutes, 4-6 times basal.

Since she had a normal response to the LHRH stimulation test and she was now more than a year without the oral contraceptive, it was decided that she should repeat her blood tests. This evaluation came out normal (Table 3).

Table 3 – Patient's results 1 year after stopping the oral contraceptive.

Parameter	Patient's results	Reference Value (RV)
Estradiol (pg/mL)	63,6	12-233 follicular phase
FSH (U/L)	4,0	3,5 – 12,5 follicular phase
LH (U/L)	2,41	2,4 – 12,6 follicular phase
Total Testosterone (ng/dL)	41,4	5-48
TSH (uU/mL)	1,66	0,51-4,3
FT4 (ng/dL)	1,43	0,9-1,63

The hypothesis of hypogonadotropic hypogonadism was then ruled out. At this time, she was transferred to the adult pediatric outpatient clinic.

It is worth noting that this patient had undergone complete development in terms of pubertal changes and stature, and had no dysmorphias. Also, although she reported acne and hirsutism, this was not clinically significant at the examination and the previous blood reports did not demonstrate hyperandrogenism.

At this point, it seemed likely that she could have an anatomical defect, though she did not have any cyclical pain. She had done a previous supra-pubic pelvic ultrasound that reported a "small uterus for the age group", but since we did not have access to this exam, a new supra-pubic pelvic ultrasound was requested. This supra-pubic pelvic ultrasound showed "a poorly individualized uterus with small dimensions (42x8mm largest diameters)". Any of these exams showed an obstructive defect or vaginal/uterine enlargement due to hematometrocolpos; however, they reported a small and poorly individualized uterus. Therefore, a pelvic magnetic resonance was requested. This exam revealed the absence of the uterus and the proximal two-thirds of the vagina (Figure 1). The uterine region was occupied by a linear structure, confirming a Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome.

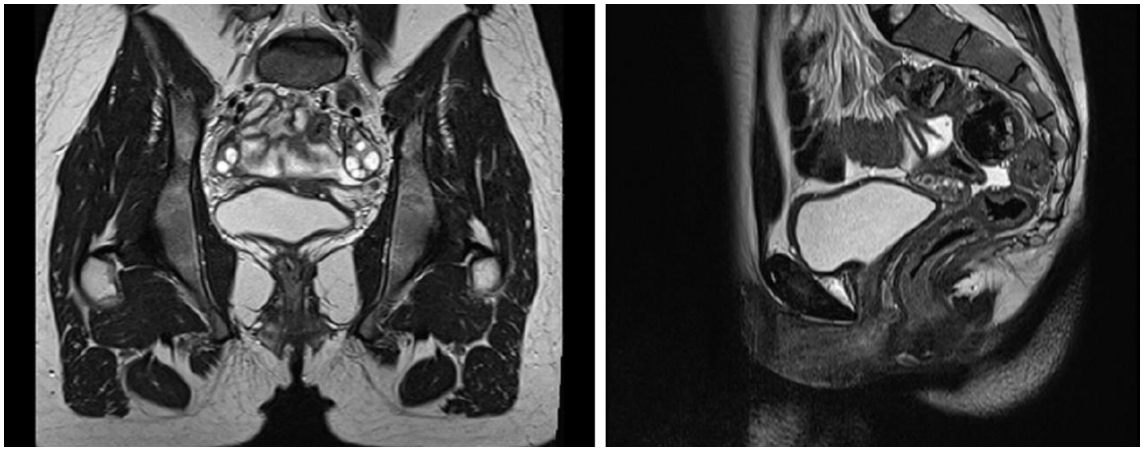


Figure 1: Pelvic Magnetic Resonance (T2-weighted imaging) demonstrating the absence of the uterus. On the left, a coronal cross-section; on the right a sagittal cross-section.

We discussed the final diagnosis with the patient and explained the impact of this malformation on her sexual and reproductive health. She was referred to an infertility clinic, where she was told she couldn't bear children but could retrieve oocytes and use a gestational carrier to have offspring. She also has a scheduled surgical gynecology appointment in the near future, but it has not occurred yet.

Finally, since this syndrome can be linked with other organ malformations, she did an abdominal CT (Computed Tomography) scan. Fortunately, this exam did not show any other malformations.

5. Discussion

The case report discusses a young woman with primary amenorrhea with MRKH syndrome, one of the many causes of primary amenorrhea.

To better understand this issue, we will now move on to reviewing the literature on the causes and management of patients with primary amenorrhea.

a) Normal gonadal and pubertal development

1) Normal gonadal development

Before discussing the causes of primary amenorrhea, it is important to know how the normal development of gonads and uterus occurs.

The chromosomal sex is established when fertilization occurs and is determined by the presence of the XX (female) or the XY (male) karyotype. For the first two months of gestation, both sexes develop identically. After that time, during the gonadal stage, the primordial gonads will develop into testicles or ovaries. The phenotypic stage is induced in response to gonadal differentiation, leading to the development of the internal genital tract and external genitalia into characteristic male or female structures. (Makiyan, 2016)

If the embryo is genetically male (XY), the primordial gonad will differentiate into testis under the influence of the SRY gene, present in the Y chromosome, which encodes the testis-determining factor. By the eighth week of gestation, Leydig cells begin to produce testosterone which contributes to the differentiation of the mesonephric ducts into the main genital ducts of the male embryo. Male sex differentiation depends on the action of testosterone on the mesonephric ducts, urogenital sinus, and external genitalia, as well as on the regression of the Müllerian ducts. (Makiyan, 2016) The mesonephric ducts will turn into seminal vesicles, vasa deferentia, and epididymides, in response to androgens. (Vue et al., 2018) In the absence of the SRY gene and testosterone, the fetus will become phenotypically female. (Makiyan, 2016)

Female embryos (XX) are SRY negative, lack testosterone, and, at this development time point, the ovaries do not secrete anti-Müllerian hormone. (Makiyan, 2016) This allows the differentiation of the Müllerian ducts into the female reproductive tract, oviducts, uterus, and upper vagina. If everything occurs flawlessly, at birth the

uterus will have a unique lumen lined with one layer of epithelial cells and surrounded by an undifferentiated mesenchyme. This will then mature into the two layers of the myometrium. (Vue et al., 2018) Then, according to the functioning of the hypothalamic-pituitary-ovarian (HPO) axis, the endometrium will suffer changes that will allow menstruation.

2) Hypothalamic - Pituitary - Ovarian (HPO) axis

Puberty is a stage of development where a child undergoes physical and psychological changes to become an adult. These changes are brought about by various endocrine systems that interact with each other and are mainly controlled by the HPO axis. (Seppä et al., 2021)

The HPO axis functions as a coordinated entity, enabling the cyclic production of steroid and gonadotropic hormones, regulating the menstrual cycle, and selecting a dominant follicle for ovulation, while also preparing the uterus for implantation. (Mikhael et al., 2019)

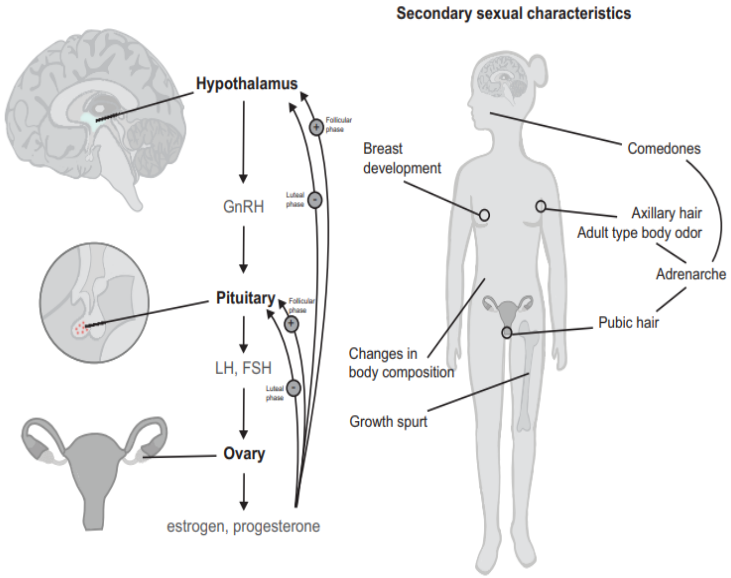


Figure 2: Hypothalamus–pituitary–ovarian (HPO) axis and secondary sexual characteristics. From Diagnosis and management of primary amenorrhea and female delayed puberty, European Journal of Endocrinology (2021) 184, R225–R242

This axis regulation begins at the hypothalamus, where neurosecretory cells produce and release gonadotropin-releasing hormone (GnRH) into the hypothalamic-

hypophyseal-portal circulation in a pulsatile form. In response to GnRH, LH and FSH are produced by the anterior pituitary and control gonadal function. (C. E. Klein, 2003) As a result, oocyte development occurs in the ovaries, and estradiol is produced by follicular growth. With follicular maturation, estradiol levels rise, LH is released, and ovulation occurs. After ovulation, the remain of the follicle becomes the *corpus luteum* and produces progesterone. If fecundation and implantation do not occur, progesterone levels decrease and the uterus sheds, which means the person menstruates and the cycle restarts. (Beshay & Carr, 2017)

Now that we have established what is necessary for menstruation to occur, we can examine the causes of primary amenorrhea.

b) Primary amenorrhea – Etiology

1) Anatomic defects (Outflow tract obstruction)

Primary amenorrhea with normal pubertal development may be caused by congenital anomalies or anatomic defects that prevent the blood from flowing monthly. (D. A. Klein & Poth, 2013) These defects may only be apparent when menstruation does not occur as it was supposed to.

1.1) Mayer-Rokitansky-Kuster-Hauser (MRKH) Syndrome

Müllerian anomalies can lead to various congenital malformations, and 7% of the female population can be affected. These abnormalities may be associated with renal and/ or anorectal congenital defects. (Verp et al., 1983)

Müllerian agenesis, or MRKH syndrome is a partial or total congenital aplasia of the uterus and the upper two-thirds of the vagina. The majority of cases are sporadic. (Verp et al., 1983)

The most common presentation of this syndrome is primary amenorrhea in a young woman with normal development of secondary sexual characters and external genitalia, as in the presented case. (Morcel et al., 2007) Other malformations, mostly renal, can be present. (Vallerie & Breech, 2010)

If Müllerian aplasia is suspected, transabdominal ultrasonography may be the first method used to diagnose it. This exam should reveal the lack of a normal uterine structure. However, a vestigial lamina underneath the peritoneal fold, transversally behind the bladder, can be mistaken for a juvenile or hypoplastic uterus. (Paniel et al., 1996) In the presented case, the diagnosis was delayed because the vestigial lamina was mistaken for a uterus. A more sensitive and specific method may be required, such as a pelvic magnetic resonance which allows better observation of the uterine and vaginal structures. (Troiano & McCarthy, 2004)

1.2) Segmental Vaginal Atresia

Adolescents with segmental vaginal atresia usually experience primary amenorrhea and cyclic abdominal pain, which is progressively more intense. Secondary sexual characteristics are well developed. (Vallerie & Breech, 2010)

It is important to do the differential diagnosis with imperforate hymen by speculum examination. (Vallerie & Breech, 2010)

1.3) Transverse Vaginal Septum

Fusion of the vaginal plate and the urogenital sinus result in transverse vaginal septum. If the septum is imperforate, the obstructed menstrual blood may cause vaginal and uterine enlargement (hematometrocolpos) and abdominal or pelvic cyclic pain. (Dennie et al., 2010)

1.4) Imperforate Hymen

The hymen is the union between urogenital sinus and sinovaginal bulbs, which is perforated during the embryonic stage. (Lee et al., 2019) When imperforated, the hymen obstructs uterine and vaginal secretions leading to cyclic pelvic pain, amenorrhea, and/ or urinary retention. (Posner & Spandorfer, 2005)

Although it is easy to diagnose by observation of external genitalia, (Lardenoije et al., 2009) sometimes the diagnosis is missed or delayed. In the abdominal ultrasound, a pelvic cystic mass may be apparent. (Lee et al., 2019)

2) Primary (Hypergonadotropic) Hypogonadism

When the ovaries are not able to produce hormones, the hypothalamic-pituitary unit doesn't receive the usual negative feedback signal, which results in increased levels of gonadotropins. (Pederson et al., 2015) Since the development of female genitalia doesn't require gonadal function and ovarian hormone production is normally low during childhood, ovarian failure or agenesis may not be detected until adolescence.

There are several causes of primary hypogonadism, including congenital (such as gonadal dysgenesis, gonadal agenesis, and enzymatic deficiencies) and acquired causes. (‘Current Evaluation of Amenorrhea’, 2008)

2.1) Gonadal dysgenesis

Gonadal dysgenesis is the atypical development of the embryo´s gonads. Girls with gonadal dysgenesis are incapable of producing normal levels of estrogen, so they will not only have amenorrhea but also very likely have underdeveloped sexual characteristics.(Breehl & Caban, 2024)

Gonadal dysgenesis can occur with normal XX and XY karyotypes, but also with abnormal karyotypes, most commonly 45, X (Turner syndrome). (Breehl & Caban, 2024)

a) Turner Syndrome

Turner syndrome is the most common genetic cause of primary amenorrhea. It is a chromosomal disorder that affects females who have one intact X chromosome and a complete or partial absence of the second sex chromosome in association with one or more clinical manifestations.(Shankar Kikkeri & Nagalli, 2024)

The physical phenotype associated with this syndrome includes short stature, webbed neck, *Pterygium colli, Cubitus valgus*, low hairline, edema of hands and feet, pigmented nevi, eyelid ptosis, and hyperconvex and/ or deep nails. Besides gonadal dysgenesis, these girls may also have heart and kidney malformations, impaired glucose tolerance, thyroid disease, and hearing loss. They usually have normal cognition but may have specific neurocognitive deficits and an increased risk of learning disabilities. This syndrome has variable presentations, so not all girls exhibit all of these features, which can result in a delay in diagnosis. (Ibarra-Ramírez & Martínez-de-Villarreal, 2016)

Turner syndrome is usually accompanied by hypergonadotropic hypogonadism and primary or secondary amenorrhea due to gonadal dysgenesis. Approximately one-third of the girls with this syndrome have spontaneous thelarche, occurring most often in girls with mosaicism. Regular menstrual cycles occur in at most 6% of these subjects. Most of these women are infertile. (Gravholt et al., 2017)

2.2) Other genetic disorders

A gene permutation of X fragile (FMR1) is associated with premature follicle atresia by the overexpression of FMR1, which downregulates oocyte development genes. This is usually more associated with secondary amenorrhea. (Chapman et al., 2015)

Girls with classic galactosemia lack enzymes to break down galactose into glucose, leading to primary ovarian insufficiency as the most common long-term complication. (Fridovich-Keil et al., 2011)

Mutations in other transcription factors responsible for gonadal differentiation and folliculogenesis such as nuclear receptor subfamily five group A member 1 (NR5A1), Newborn Ovary Homeobox (NOBOX), and Factor in Germline alpha (FIGLA) can also be responsible for ovarian insufficiency. (Chapman et al., 2015)

2.3) FSH or LH receptor Mutations

Ovarian failure can also be caused by mutations of FSH or LH receptors. Mutations can translate into a gain or loss of function of the receptor. (Breehl & Caban, 2024)

Loss of function mutations of LH receptor lead to hypergonadotropic hypogonadism. Since the receptor is not functioning properly, levels of LH are elevated, and FSH can be normal or slightly elevated. Since the ovaries cannot sense the LH stimulus, female patients present amenorrhea or oligomenorrhea, but normal breast and pubic hair development. (Breehl & Caban, 2024)

2.4) Iatrogenic and Acquired causes

Iatrogenic and acquired causes of primary hypogonadism are more commonly associated with secondary amenorrhea since they usually occur later in life. These may include sequelae of oncologic treatments (radio and/ or chemotherapy), surgeries, trauma, and autoimmune oophoritis which can be associated with other autoimmune diseases like Hashimoto Thyroiditis, Addison's Disease and Type 1 Diabetes Mellitus. (Mikhael et al., 2019)

3) Secondary (Hypogonadotropic) Hypogonadism

Central causes related to the hypothalamus and/or pituitary gland can be classified into acquired, congenital, or functional disorders. (‘Current Evaluation of Amenorrhea’, 2008)

The most common cause of delayed growth and puberty is the late activation of the HPO axis. This delay is known as constitutional delay of growth and puberty (CDGP), and it's often seen in families with a history of CDGP. Children with CDGP may be shorter than their peers due to delayed bone development, but they will achieve normal development milestones at a normal bone age. Laboratory results will show prepubertal FSH and LH concentrations. (Seppä et al., 2021)

3.1) Acquired Disorders

Acquired disorders causing hypogonadotropic hypogonadism can be related to tumors, infections, surgeries, radiotherapy, infiltrative diseases, trauma, pituitary apoplexy, and vascular lesions. These may lead to pituitary gland or stalk damage. Importantly, these disorders can be associated with combined pituitary deficiencies, so other symptoms may be apparent, like short stature (due to growth hormone deficiency). (Majumdar & Mangal, 2013)

Prolactinomas, which are the most common type of pituitary tumor, not only cause amenorrhea due to the mass effect but also due to the suppression of pulsatile GnRH by prolactin (PRL). The overproduction of PRL can also cause galactorrhea. (Majumdar & Mangal, 2013)

3.2) Congenital Disorders

Central hypogonadism can also be congenital and appear isolated or combined with other pituitary deficiencies. Mutations in more than thirty genes have been associated with congenital GnRH deficiency, leading to hypogonadotropic hypogonadism, which may be accompanied by anosmia or hyposmia (Kallmann's syndrome). Mutations in the FSH beta subunit (FSHβ) gene are rare and associated with the absence of sex characters and primary amenorrhea. Mutations in the LH beta subunit (LHβ) gene are also rare. Mutations in the PIT1 prophet gene (PROP1),

a pituitary transcription factor, lead to combined deficiencies of pituitary hormones such as TSH, PRL, growth hormone (GH), and gonadotropins. (Layman, 1999) Other syndromes like Prader–Willi syndrome, Laurence–Moon syndrome, and Bardet–Biedl syndrome are also frequently associated with central hypogonadism. (Hypogonadism Clinical Presentation: History, Physical, Causes, n.d.)

3.3) Functional Disorders

Low energy availability caused by decreased caloric intake, excessive energy expenditure, or both can lead to hypogonadotropic hypogonadism or functional hypothalamic amenorrhea. This can also be caused by stress, psychiatric illness, or chronic disease (e.g. celiac disease, inflammatory bowel disease). These disorders primarily affect the pulsatility of GnRH, (Mikhael et al., 2019) which leads to low levels of FSH, LH, and estradiol. They are often associated with secondary amenorrhea, but can also cause primary amenorrhea. (Shufelt et al., 2017)

4) Other endocrine disorders

Patients with amenorrhea may have normal estrogenic levels. In this situation, estrogen is secreted acyclically by the ovaries, compromising the retrocontrol over the hypothalamic-pituitary axis. Chronic anovulation with normal estrogen can occur in the presence of other endocrine pathologies, such as adrenal or ovarian tumors, thyroid disease, Cushing's syndrome, and Congenital Adrenal Hyperplasia (CAH). (Anovulation, 2023)

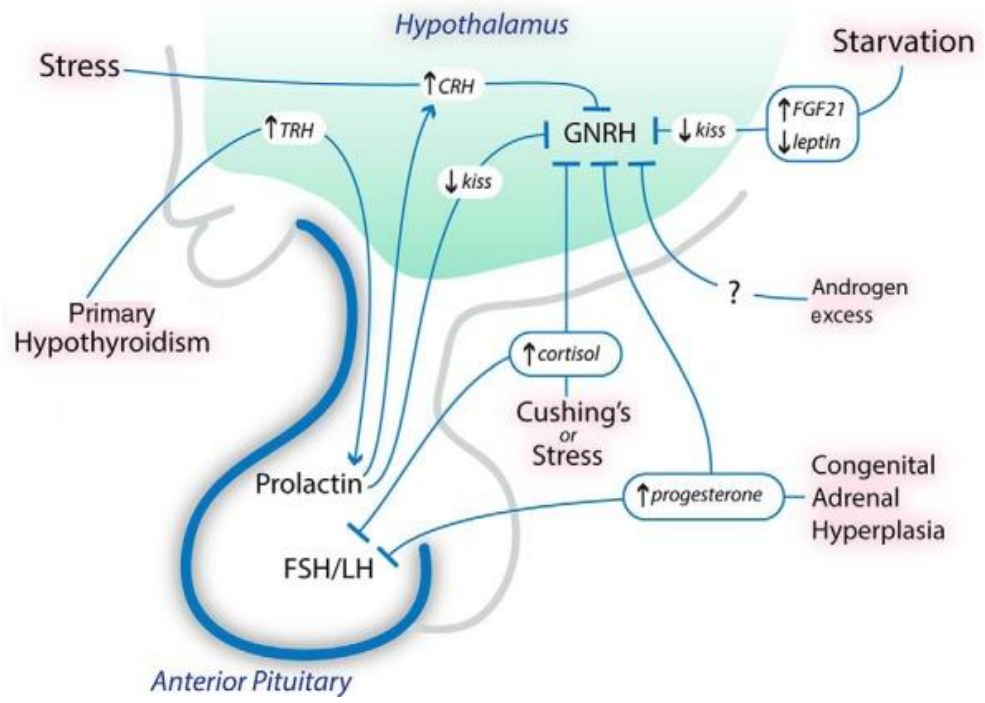


Figure 3: Potential hormonal mediators of amenorrhea. From Lindsay T. Fourman, Pouneh K. Fazeli, Neuroendocrine Causes of Amenorrhea—An Update, The Journal of Clinical Endocrinology & Metabolism, Volume 100, Issue 3, 1 March 2015, Pages 812–824

5) Other rare, non-endocrine, causes

Amenorrhea can occur in genetically male individuals who appear female phenotypically, due to Adrenal Insensitivity Syndrome (AIS) and 5-alpha-reductase deficiency. These individuals may have a predominantly female phenotype and normal female external genitalia. However, they lack internal female genitalia, so, at puberty, they will have amenorrhea. (Kumar & Barboza-Meca, 2024; Makiyan, 2016)

These conditions are rare and should be apparent once the karyotype is done (46, XY).

6) Causes of Primary Amenorrhea – Summary table

Anatomic Defects	• MRKH Syndrome • Segmental Vaginal Atresia • Transverse Vaginal Septum • Imperforate Hymen	

Hypergonadotropic Hypogonadism	• Gonadal Dysgenesis
	  ○ Normal Karyotype
	  ○ Abnormal Karyotype
	  ▪ Turner Syndrome
	• Other Genetic defects
	  ○ Permutation X Fragil
	  ○ Galactosemia
	• FSH / LH receptor mutations
		• Iatrogenic and Acquired causes
		  ○ Chemo and/or Radiotherapy
		  ○ Surgery
		  ○ Trauma
		  ○ Autoimmune oophoritis

Hypogonadotropic Hypogonadism	• Constitutional delay of growth and puberty
	• Acquired disorders
	  ○ Tumors
	  ▪ Prolactinoma
	  ○ Surgery and / or Radiotherapy
	  ○ Trauma
	  ○ Infections
		• Congenital disorders
		  ○ Isolated GnRH deficiency
		  ▪ Kallman Syndrome
		  ○ Combined deficiencies
		• Functional disorders
		  ○ Low caloric intake, excessive exercise
		  ○ Chronic disease

Other endocrine disorders	• Hyperandrogenism
	  ○ Tumors
	  ○ CAH
		• Hypercortisolism
		  ○ Cushing´s Syndrome
		• Thyroid disease

Other rare causes	• Androgen Insensitivity Syndrome	• 5 alpha-Reductase

c) Approaching Primary Amenorrhea Patients

The diagnostic approach must be guided by the history and physical examination.

1) Anamnesis

To determine the cause of primary amenorrhea, a thorough medical examination, including a medical history, physical examination, and laboratory analysis, is necessary. (D. A. Klein & Poth, 2013)

When taking a medical history, it is important to consider the timing of breast and pubic hair development, eating and exercise habits, and any history of social or psychological distress. It is also important to rule out chronic diseases and treatments, especially exposure to chemotherapy or radiotherapy. (D. A. Klein et al., 2019; D. A. Klein & Poth, 2013; Seppä et al., 2021)

Additionally, it is important to ask about the age of menarche among close relatives, as well as the timing of puberty onset for parents and siblings. Other relevant questions include any history of chronic diseases and midline abnormalities. (Seppä et al., 2021)

It is also necessary to inquire about any other relevant symptoms that may help with clinical reasoning, such as headaches, nausea, vision disorders, galactorrhea, anosmia/hyposmia or cyclical abdominal pain. (Seppä et al., 2021)

2) Physical Exam

It is crucial to obtain accurate information about the patient's height, weight, and body mass index, as well as their growth charts. During the physical examination, it is important to perform a comprehensive and systematic evaluation. This evaluation should include an assessment of the breasts and external genitalia, and they should be classified according to the Tanner staging. The development of breasts is an excellent indicator of estrogen production. The hair distribution should also be examined. Abdominal examination and palpation can also be useful. (D. A. Klein et al., 2019; D. A. Klein & Poth, 2013; Seppä et al., 2021)

It is important to remain alert to other signs and symptoms that may be indicative of underlying conditions. Hirsutism and acne may be signs of hyperandrogenism, whereas striae, moon face, buffalo hump, and central obesity may be signs of hypercortisolism. Eating disorders may be suspected in cases where the patient has a low body mass index and presents with lanugo, cold extremities, and bradycardia. However, it is important to note that these signs may not always be present. (Seppä et al., 2021)

If a patient presents with short stature, particularly if they have distinctive features such as a webbed neck and low hairline, it is crucial to exclude any karyotype anomalies. (Seppä et al., 2021)

A pelvic examination is a crucial part of the physical exam and can detect the absence or anomalies of the cervix or uterus. It can identify transverse septum or imperforate hymen, both of which are outflow tract obstructions. (D. A. Klein & Poth, 2013)

3) Amenorrhea with normal secondary sexual characters

If a girl has normal pubertal development but no menarche, it is important to check for the presence of the uterus through a pelvic ultrasound or magnetic resonance, which can also reveal any structural abnormalities in the reproductive tract organs. (Seppä et al., 2021)

If the uterus is present, blood might be seen accumulated in the uterus or vagina, in the pelvic imaging exams, meaning an outflow tract obstruction, such as imperforate hymen or transverse vaginal septum, for example. (Seppä et al., 2021)

If the pelvic imaging exam shows a normal uterus and the patient has signs of hyperandrogenism, it may be useful to request blood tests including total testosterone, Dehydroepiandrosterone (DHEA) or Dehydroepiandrosterone Sulfate (DHEA-S), 17-hydroxyprogesterone and androstenedione levels. (Seppä et al., 2021)

If the uterus cannot be identified, it is important to request a karyotype. If the karyotype shows a normal count of 46,XX chromosomes, the patient likely has Müllerian agenesis or MRKH Syndrome. On the other hand, if the karyotype reveals

a male count of 46, XY chromosomes, it can be an Androgen insensitivity syndrome. (Seppä et al., 2021)

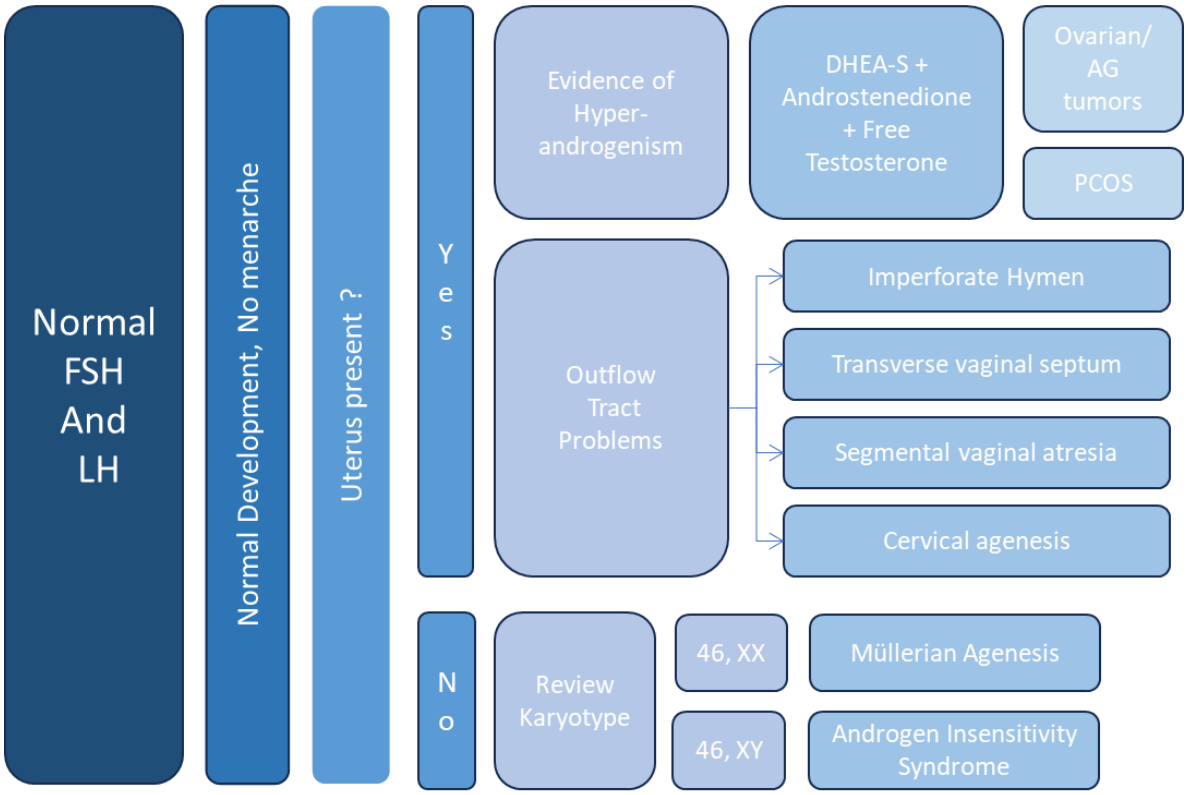
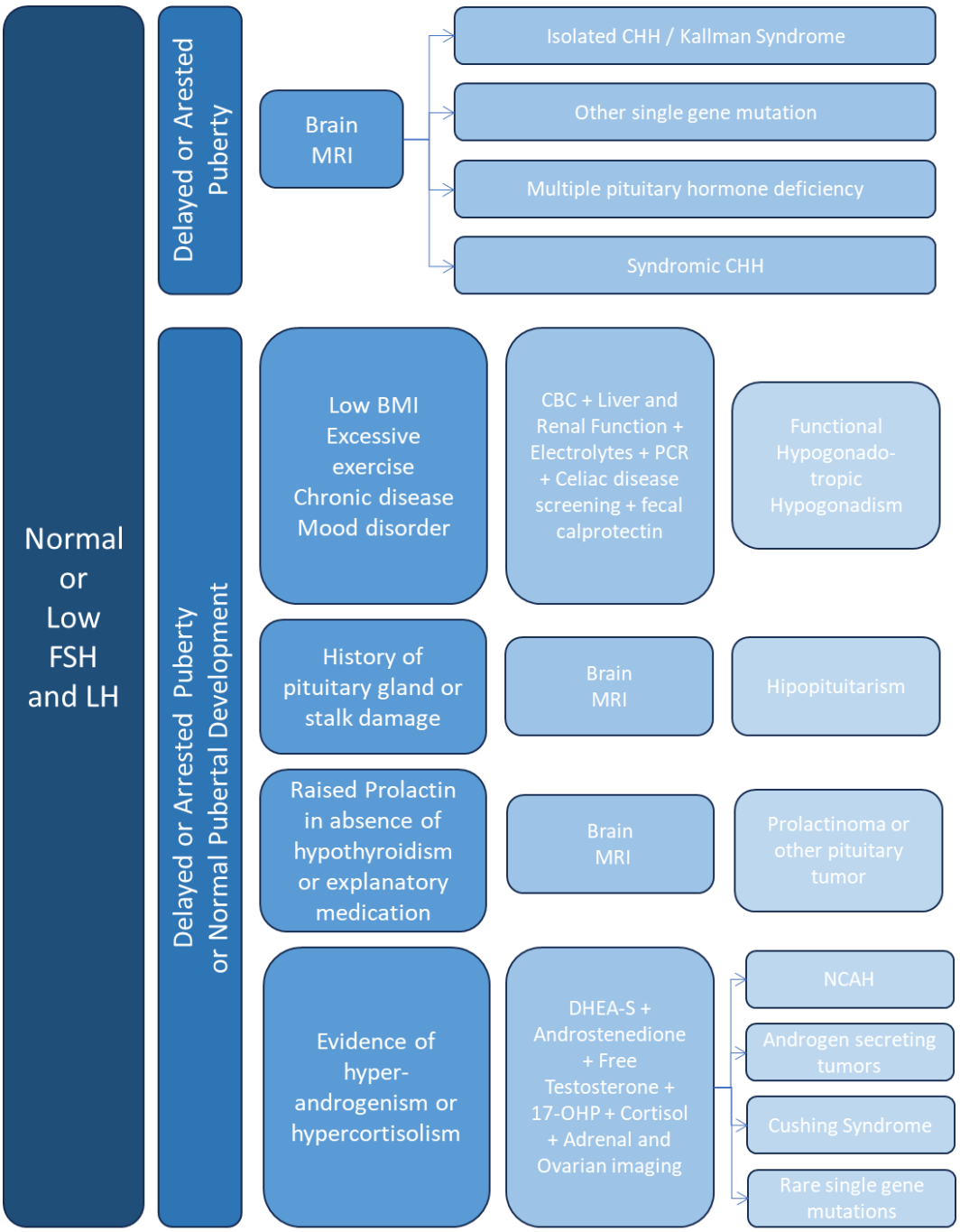


Figure 4: Algorithm for the approach to the patient with primary amenorrhea with normal secondary sexual characteristics based on Figure 2 of Diagnosis and management of primary amenorrhea and female delayed puberty, European Journal of Endocrinology (2021) 184, R225–R242 (DHEA-S- Dehydroepiandrosterone Sulfate; PCOS- Polycistic Ovarian Syndrome; AG- Adrenal gland.)

4) Amenorrhea without normal secondary sexual characters

Girls with amenorrhea without normal secondary sexual characteristics may have hypogonadism or constitutional delay of puberty. If hypogonadism is more likely, it should be characterized according to the level of FSH and LH, in hypogonadotropic (secondary) or hypergonadotropic (primary). (Seppä et al., 2021)



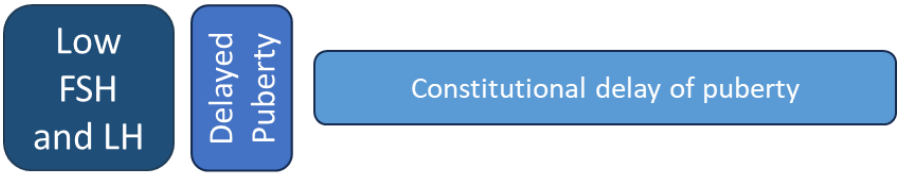


Figure 5: Algorithm for the approach to the patient with primary amenorrhea without normal secondary sexual characteristics based on figure 2 of Diagnosis and management of primary amenorrhea and female delayed puberty, European Journal of Endocrinology (2021) 184, R225–R242 (17-OHP- 17-hydroxyprogesterone; BMI– Body Mass Index; CBC- Complete blood count; CHH - Congenital hypogonadotropic hypogonadism; DHEA-S- Dehydroepiandrosterone Sulfate; MRI- Magnetic Resonance Imaging; NCAH- Non-classic congenital adrenal hyperplasia.)

If the levels of FSH and LH are low, a cranial magnetic resonance might be useful to know the central cause of this hypogonadism. (Seppä et al., 2021)

If the levels of FSH and LH are high, a karyotype is mandatory. (Seppä et al., 2021)

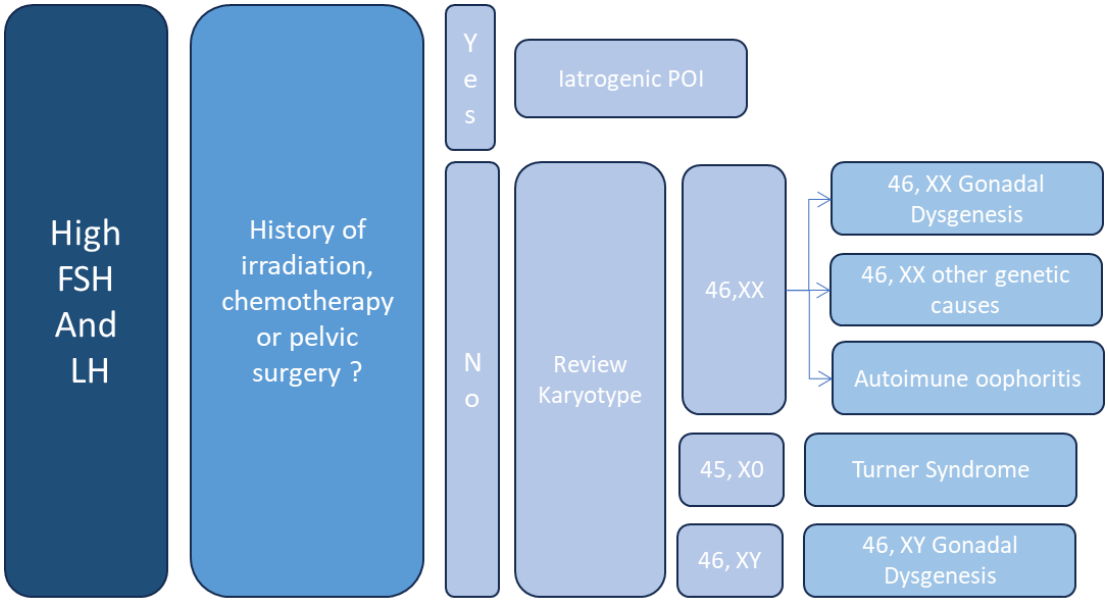


Figure 6: Algorithm for the approach to the patient with primary amenorrhea without normal secondary sexual characteristics and hypergonadotropic hypogonadism based on figure 2 of Diagnosis and management of primary amenorrhea and female delayed puberty, European Journal of Endocrinology (2021) 184, R225–R242 (POI- Primary Ovarian Insufficiency)

6. Conclusion

Menstruation serves as a crucial indicator of overall health and well-being, with irregular patterns or absence of menses potentially signaling underlying health issues.(D. A. Klein et al., 2019) This work focused on primary amenorrhea. Various conditions can manifest as amenorrhea, requiring a comprehensive investigation to avoid overlooking significant diagnoses. (‘Current Evaluation of Amenorrhea’, 2008)

The case report shown at the beginning pretends to highlight the diagnostic challenges encountered in managing primary amenorrhea. Primary amenorrhea in young people with adequate pubertal development and hormonal profile should guide the exclusion of structural pathology of the uterus/vagina. In the presented case, the initial laboratory evaluation, done shortly after stopping the oral contraceptive, suggested an unlikely diagnosis. A more detailed examination, however, made it possible to reach a diagnosis. This case illustrates that the clinical sense must prevail, especially when complementary means of diagnosis do not offer satisfactory answers.

Approaching patients with primary amenorrhea requires a comprehensive evaluation including a complete medical history, physical examination, and laboratory analysis. Diagnostic modalities such as pelvic ultrasound, magnetic resonance, and CT imaging may help us reach to a final diagnosis. (Seppä et al., 2021)

In conclusion, since there are multiple causes of primary amenorrhea, it can be challenging to diagnose its cause. This emphasizes the importance of a systematic approach to the etiology of primary amenorrhea. After the diagnosis, it is important to treat the underlying cause, if possible, and address possible complications, which may benefit from a multidisciplinary approach, involving different medical or surgical specialties.

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