



Meet the Expert HANDOUTS

These sessions are interactive.
Seats are limited and will therefore be allocated on a
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Statements on any potential conflict of interest will be shown by the
speaker at the beginning of their session.

Opinions and recommendations made by the
presenters are not those of ESPE.



Meet the Expert Session
1.1 & 1.2

Clinical management of transgender
youth

Olle Söder (Stockholm, Sweden)

Saturday 10 September 16:15 – 17:15 – 252 AB

Monday 12 September 08:00 – 09:00 – Amphithéâtre Bordeaux

Clinical management of transgender youth

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Introduction

Gender dysphoria (DSM-5) or transsexualism (ICD-10) is defined as a persistent desire to live and be accepted as the opposite sex, usually accompanied by a perceived inconsistency with the natal sex and a desire to change the body in accordance with the perceived sex (1,2). The underlying biology and etiology of the disorder are unknown despite recent attempts to etiological mapping including genetic analyses, endocrine studies and use of modern brain imaging techniques.

Most youths who seek help for gender dysphoria can tell about an early onset ("as long as I can remember") which is supported by the parents, referring to play behavior, clothes choice, etc. Approximately one third has onset in early puberty. Studies have shown that there is only a minority (about 20 %) of prepubertal children with gender dysphoria, who will retain a persistent desire for gender reassignment as a young adult. In contrast, a majority of those whose gender dysphoria reinforced during puberty and adolescence will later meet the diagnostic criteria for gender dysphoria and transsexualism (3,4). They may be offered treatment with puberty stopping hormones to avoid the development of undesired secondary sexual characteristics.

In contrast to adults (5) there is little population based data on the prevalence of gender dysphoria in children. However, in the past few decades there has been a dramatic increase in the number of young people seeking medical help for gender dysphoria asking for gender-confirming medical interventions (Fig. 1). Interestingly and opposite to adults, the majority of young people with gender dysphoria are female-to-male (FtM). Thus, in our youth clinic referrals between 2013-2015 hosted 75% FtM and 25% male-to-female (MtF) subjects. This should be compared with the increasing prevalence in Swedish adults with a majority of MtF (5). The reasons for this recent increase in young people with gender dysphoria and the skewed gender balance are unknown.

Diagnosis of gender dysphoria in youths

Gender dysphoria in children, as in adults, is regarded as a psychiatric diagnosis to be set by a psychiatric specialist team with special competence and experience of child psychiatry, following accepted principles (1,2). Several potential differential diagnoses may exist in children and must be ruled out. These include identity crisis in adolescence, emerging mental disorder (psychosis), confusion with sexual orientation (pre-homosexuality), cryptic disorder of sex development (DSD; e.g., 5-alpha reductase deficiency) and others. Typically, these conditions are already recognized in young adults but may not be so in young children and teenagers. Neuropsychiatric co-morbidities are common (see separate section) and must be considered and may need separate care. We have also experienced factitious situations with subjects expressing gender dysphoria, later revealed to be made up due to conflict with parents. Such situation is not easy to disclose as there is no objective independent way of setting the diagnosis. The diagnostic tools available depend solely on the communicative skills of the subject. The diagnostic criteria are well known also in lay literature and may be "borrowed" for use as above. In our pediatric endocrinology setting first visit referrals for gender dysphoria in children are accepted only if the diagnosis is considered highly probable after thorough work-up by a child psychiatric specialist team.

Work-up and care by child psychiatrist/psychologist

Gender-confirming medical intervention is the only recommended treatment for gender dysphoria. Early treatment facilitates the ability to successfully pass as the desired sex and this is associated with a significantly better prognosis. The child psychiatrist should be principal physician in charge of the diagnostic work-up to set the gender dysphoria diagnosis and to exclude differential diagnoses. The psychiatric team should be responsible for psychological support and networking with other medical specialties such as pediatric endocrinology, reconstructive surgery, speech therapy, gynecology and others. The plastic surgeon is important to explain the surgical techniques that are available and their results and is also responsible for performing mastectomy in FtM patients. The principal physician should also be responsible to help the patient with the formal application to the authorities for gender re-assignment.

Work-up and endocrine treatment by the paediatric endocrinologist

The pediatric endocrinologist should be responsible for the somatic work-up including medical background, physical examination and laboratory tests. He/She should record the present and past medical history and developmental milestones, especially pubertal development and present status by Tanner staging. It is important to exclude cryptic DSD although this seems very rare. We have seen one single such case (undiscovered 5-alpha reductase deficiency) in the past 30 years. The laboratory work-up should include gonadotropins, sex steroids, adrenal androgens, karyotype and vitamin D status.

Invariably, gender dysphoria patients referred to pediatric endocrinology ask for “stop hormones” (i.e., GnRH analogs) to block their unwanted puberty. Our policy is to offer such treatment provided certain criteria are fulfilled. These are; 1) A strong wish for treatment; 2) Diagnostic work-up by specialist psychiatry team has demonstrated that gender dysphoria/transsexualism is highly “probable”; 3) Pubertal stage is at least Tanner 2. We do not offer GnRH analog treatment to prepubertal children as exposure to their endogenous sex steroids is used as a diagnostic tool. At this phase of work-up most subjects are in successful “real life experience”, practicing the social life of the desired sex. This should be closely monitored by the psychiatry team as failure should result in reconsideration of the diagnosis. Withdrawal symptoms (as in postmenopausal women) are common adverse side effects during treatment with GnRH analogs. They usually disappear after start of treatment with sex steroids. Long-term GnRH analog treatment without addition of sex steroids may negatively affect the future bone health. Vitamin D and calcium status should therefore be closely monitored and vitamin D prophylaxis installed.

After further work-up cross sex steroids may be offered to induce puberty in the wanted sex. Such treatment will cause irreversible effects (e.g., male voice pitch in FtM and breast development in MtF) and we therefore practice stricter criteria: 1) Strong wish for treatment; 2) “Definitive” diagnosis of gender dysphoria by specialist psychiatry team; 3) Age 16 or older. We use increasing doses of testosterone by i.m. injection to FtM and estrogen patches to MtF. The GnRH analog treatment is maintained to securely that the endogenous hormonal secretion is silenced. There is no need for progesterone. The treatment principles and choice of pharmaceuticals with GnRH analogs (7) and cross sex steroids are the same as for treatment of precocious puberty and induction of male or female puberty in cases of pubertal delay. These principles are well recognized by the pediatric endocrinologist and not further discussed here. Acne and elevated hemoglobin concentration are common side effects of testosterone treatment. Estrogen increases the risk of thrombo embolic disease although such complication is still rare.

From age 18 transition to adult endocrinology is possible and should be facilitated by the pediatric endocrinologist. The pharmacological treatment of adults with gender dysphoria may differ from the practice in children and adolescents.

Fertility preservation

Previously Swedish law did not permit gender reassignment unless the subject was made irreversibly sterile, usually by gonadectomy. The law was changed in 2013 and from then allowing measures to preserve fertility such as gamete and gonadal tissue cryopreservation or more conservative (or no) genital surgery. Presently patients and parents are informed about this possibility fertility preservation and if of interest they are referred to the reproductive medicine division for further discussion. However, some patients declare firmly that they have no interest of such issue. Before any invasive measures are taken in reproductive medicine infectious diseases such as HIV and hepatitis need to be excluded and such tests are to be managed by the pediatric endocrinology clinic.

Speech Therapy

All gender dysphoria patients are offered a visit to our speech therapy team which has set up specialist care for this group. Treatment is usually offered MtF patients as described (6).

Co-morbidity with neuropsychiatric disorders in youths

A majority of young people with gender dysphoria expresses signs of psychiatric illness (8), most commonly anxiety, depression and self-destructive behavior (9) . Published data refer to a prevalence of 26% of autism spectrum disorders (AST) in this group (10). Our experience is that AST and attention disorders may be much more common although a formal diagnostic work-up is lacking.

Prognostic factors

The most important prognostic factor in gender dysphoria is to "pass" as a member of the desired sex. The prognosis after sex re-assignment is therefore much better if puberty stopping treatment has been given as this has reduced the development of unwanted secondary sex characteristics. A well- functioning social network is also important including support from the family (parents in young people). A recent Swedish follow-up study with adults demonstrates fewer regrets in recent years, indicating improved work-up and treatment (3). We still await similar data after long term follow –up of youths with gender dysphoria.

Disclaimer

The views expressed in this text are aimed for a Meet the Expert session at the ESPE Annual Meeting in Paris 2016 and is mainly based on the clinical experience of the author. The evidence base in this area is still weak. The reader should be aware that the legal framework for diagnosis and treatment of young people with gender dysphoria may differ in different countries in Europe and world-wide. Recommendations of unlabeled use of pharmaceuticals may occur. The author has no conflict of interest.

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Bulleted Facts for Gender Dysphoria in Youths

- Gender dysphoria or transsexualism is defined as a persistent desire to live and be accepted as the opposite sex, usually accompanied by a perceived inconsistency with the natal sex and a desire to change the body in accordance with the perceived sex.
- Many centers world-wide have experienced a recent dramatic increase of youths seeking medical help for gender dysphoria. A majority of these subjects are FtM which is in contrast to adults with gender dysphoria.
- Most youths with gender dysphoria refer to an early start; from preschool age or earlier. Follow-up studies have shown that only a minority (<20%) of younger children (<12 years of age) sustain their wish for gender reassignment as young adults.
- In contrast, the majority of those who express and deepen their gender dysphoria during puberty fulfill the diagnostic criteria of transsexualism as young adults.
- Specialized child psychiatrists/psychologists should be responsible for the work-up and diagnosis of gender dysphoria in youths. Pediatric endocrinologists should be consulted for endocrine work-up and treatment with GnRH analogs and cross sex steroids.

- Early start of endocrine treatment facilitates the possibility to pass in the desired sex, which is associated with a better long term prognosis.

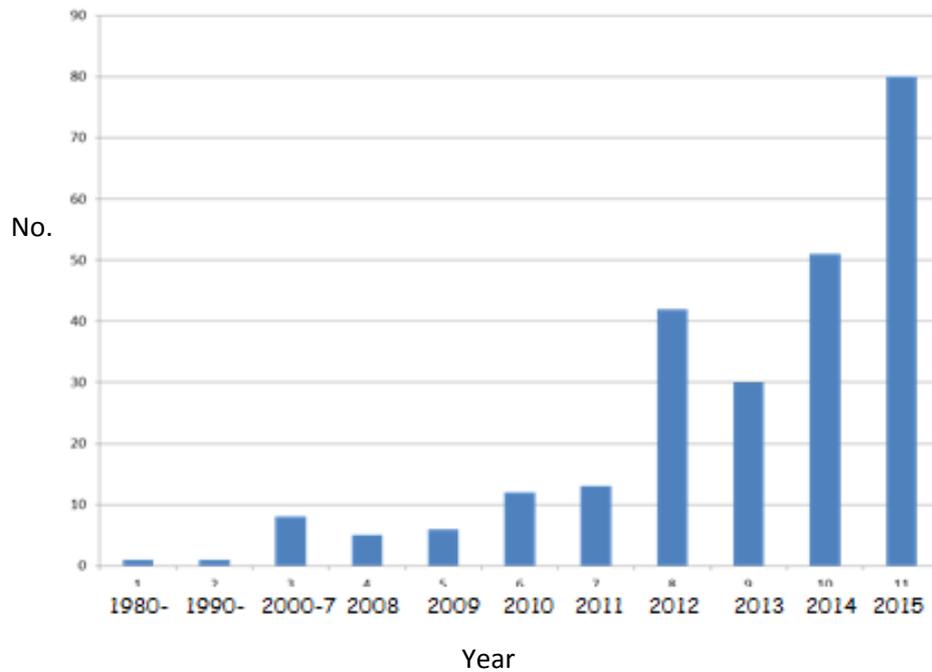


Figure 1. Number of referrals of children and adolescents (age <18 y.) with gender dysphoria to Karolinska center 1980-2015



Meet the Expert Session
2.1 & 2.2

Hypertension in children

Jörg Dötsch (Cologne, Germany)

Saturday 10 September 16:15 – 17:15 – 342 AB

Sunday 11 September 08:00 – 09:00 – Amphithéâtre Bleu

Hypertension in Childhood

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Introduction

Arterial Hypertension is a topic with rising interest. As a consequence of the increasing number of obese children and adolescents, hypertension becomes more prevalent in this age group. In addition, recent adult data indicate that blood pressure even in the normal range may predispose to cardiovascular disease (Wright et al., 2015). Whether this is of importance in children and adolescents already is still an issue to be discussed. However, there is increasing evidence that patients with high blood pressure in adulthood already were affected in younger years (Falkner and Gidding, 2016): The relationship between childhood BP and BP status in young adulthood was further delineated in an analysis of data from the Dunedin Multidisciplinary Health and Development Study, a prospective cohort study that included periodic BP and other risk factor measurements from age 7 to 38 years. Four distinct BP trajectory groups were identified according to BP status at age 38 years; normal, high-normal, prehypertensive, and hypertensive. The hypertensive trajectory had the highest BP levels in childhood, and the prehypertensive trajectory had the next highest childhood BP levels, with systolic BP levels above 120 mm Hg in adolescence. For those with normal and

high-normal BP at age 38 years, systolic BP throughout childhood and adolescence was below 120 mm Hg. An increase in body mass index was found to be significantly associated with an upward shift in all 4 BP trajectory groups (Falkner and Gidding, 2016, Theodore et al., 2015).

These novel studies clearly demonstrate that the management of hypertension in childhood and adolescents may not only be of importance in the context of the search for a potentially underlying disease but also to prevent consequences in adulthood on a large scale. The following Mini-review will cover the questions of appropriate blood pressure measurements, aetiology, and treatment.

Definitions and Methodology of Blood Pressure Measurements

On the first glance this appears trivial. However, first of all it has to be born in mind that in the outpatient setting we apply different measurement methods, i.e. the traditional auscultatory (Riva-Rocci) method and the oszillometric measurement. While the first determines systolic and diastolic blood pressure, the latter measures mean arterial pressure. Systolic and diastolic blood pressure is calculated. Finally, ambulatory blood pressure measurements (ABPM), though oszillometric, has completely different reference values than the office measurement values (Dötsch and Rascher, 2014).

Currently, two items are of particular interest in the literature. One is the item of masked hypertension, i.e. hypertension that is not picked up by office measurements but only by ABPM or the search for end organ damage. However, recently it could be shown that office blood pressure measurements are also higher when masked hypertension is present (Mitsnefes et al., 2016).

An even more challenging question is how often casual blood pressure measurements should be repeated before white coat hypertension becomes unlikely. A recent study could demonstrate that assessment of blood pressure using oscillometric devices should include at least 3 measurements in the same sitting to avoid inaccurate assessment (Negróni-Balasquide et al., 2016).

It should be born in mind, however, that a major issue of relevant blood pressure throughout all stages of life is end organ damage. Among the organs affected retinopathy, renal damage and increasing left ventricular mass are the most important throughout childhood. While hypertensive retinopathy is not always easy to diagnose, renal damage may only appear at a relatively late stage as a consequence of the large compensatory capacity of the kidneys. Left ventricular mass is easily accessible and therefore plays a pivotal role in the diagnosis of end organ damage. It is quite interesting, however, that obesity, one major risk factor for hypertension is a risk factor for left ventricular hypertrophy independently from blood pressure itself (Ramaswamy et al, 2016).

Aetiology of hypertension

Traditionally hypertension in childhood was mostly attributed to secondary causes. This has, however, changed. Nowadays, the prevalence of primary and secondary hypertension is equal around the age of school entry. However, it is still arguable, whether hypertension associated with obesity is rather secondary or primary. Leaving obesity out of the secondary hypertension causes, renal parenchymal disease is still by far the commonest cause, followed by cardiovascular, endocrine, and renovascular aetiologies.

Nonetheless, the causes of hypertension in childhood and adolescence differ tremendously throughout the age groups (Patel and Walker, 2016). Especially in the newborn era diseases associated with classical neonatal or preterm newborn disease are found that later on have no more role (e.g. renal vein thrombosis and bronchopulmonary dysplasia).

Three items relevant to the endocrinologist will be addressed in this min-review: the so called **monogenic forms of hypertension, obesity related**, and **developmental origins of hypertension**. Most of the monogenic causes of hypertension are rare. Of special interest to the endocrinologist are potentially the forms of congenital adrenal hyperplasia associated with hypertension, i.e. 11 β -hydroxylase and 17-hydroxylase deficiency. These must not be confused with the non-hypertensive form that develops high blood pressure as a

consequence of long lasting medication with glucocorticoids and mineralocorticoids (Maccabee-Ryaboy et al., 2016).

Other monogenic forms are apparent mineralocorticoid excess (AME), caused by 11 β -hydroxysteroid dehydrogenase deficiency, glucocorticoid remediable aldosteronism (GRA), which is caused by a chimeric aldosterone gene that has come under the control of ACTH, and Liddle's syndrome, inflicted by an activation mutation of the epithelial sodium channel which leads to more sodium reabsorption (Toka et al. 2013).

The mechanisms of obesity associated hypertension have become quite clear in the last few years. Major factors are endothelial dysfunction, inflammation, activation of the renin-angiotensin-aldosterone system, and sympathetic nerve system activation (Wirix et al., 2015). One more aspect in the aetiologic role of obesity induced hypertension is the observation, that a BMI-SDS reduction > 0.25 significantly improves hypertension (Reinehr et al., 2016).

One interesting aspect of hypertension genesis is the field of perinatal programming. Low birth weight as well as high birth weight has been associated with hypertension later in life. Early weight gain also predisposes to higher diastolic blood pressure levels in childhood. More recently, family structure as such has been linked to childhood hypertension. For example, single parent status is associated with a higher prevalence, while higher education and parent employment are linked to a lower prevalence of hypertension (Gupta-Malhotra et al., 2016).

Treatment

Treatment may be divided into the subchapters of principal treatment, treatment in special disease situations, and hypertensive crisis. In the last 10 years numerous studies have been published examining safety and dosing of antihypertensive medication. A summary of drugs commonly used is shown in Table 1.

Conclusion

Hypertension in Childhood and Adolescence has gained interest in the last couple of years. This may be attributed to an increasing importance with regard to adult cardiovascular disease, emerging new causes and conclusive treatment studies.

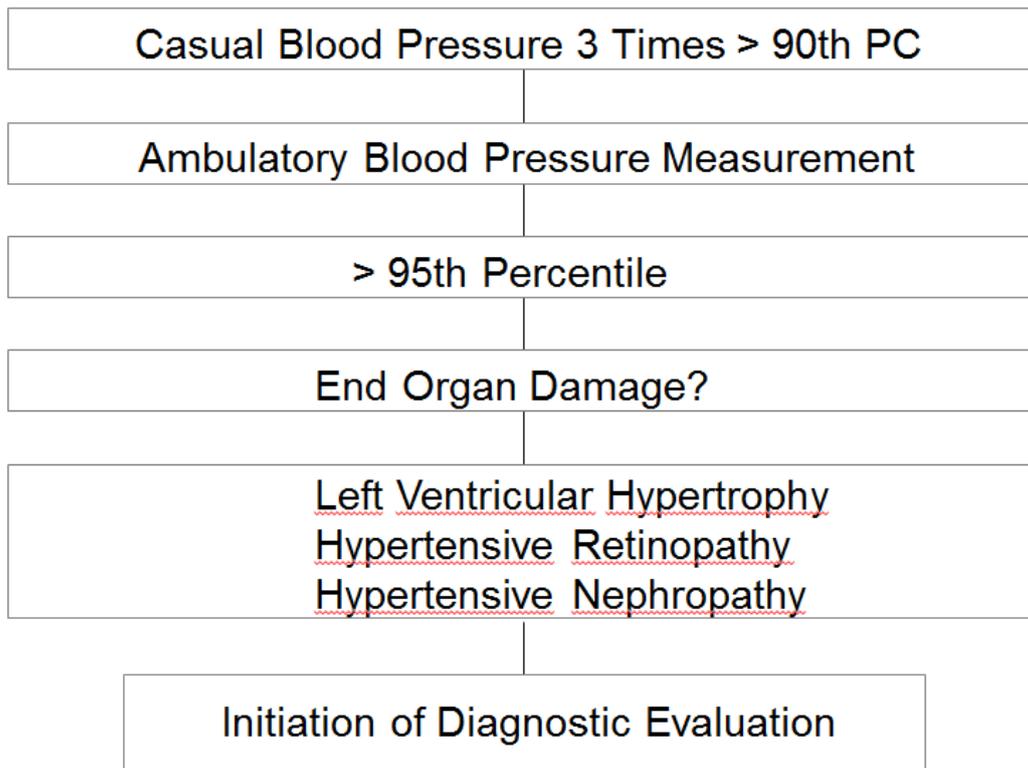
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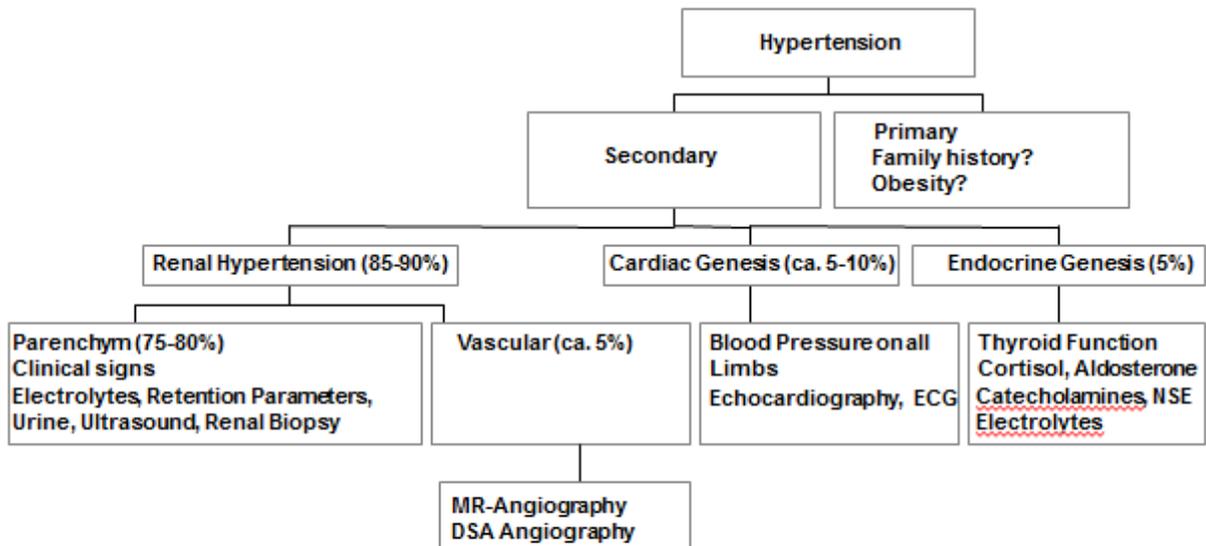
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Figure 1: Algorithms for the Evaluation of Hypertension in Childhood

Algorithm Hypertension



Algorithm Hypertension (2)





Meet the Expert Session
3.1 & 3.2

Glucocorticoid induced fractures

Outi Mäkitie (Stockholm, Sweden)

Saturday 10 September 16:15 – 17:15 – Salle Maillot
Monday 12 September 08:00 – 09:00 – Salle Maillot



Meet the Expert Session
4.1 & 4.2

Neonatal Graves disease

Juliane Leger (Paris, France)

Saturday 10 September 16:15 –17:15 – Amphithéâtre Bordeaux
Monday 12 September 08:00 – 09:00 – Amphithéâtre Bleu

Management of Foetal and Neonatal Graves' Disease

Juliane Léger

Paediatric Endocrinology Department, Assistance Publique-Hôpitaux de Paris, Hôpital Universitaire Robert Debré, Centre de Référence des Maladies Endocriniennes Rares de la Croissance, Université Paris Diderot, Sorbonne Paris Cité, F-75019 Paris, France

Two clinical cases will be discussed in detail, in an interactive form.

Neonatal autoimmune hyperthyroidism (neonatal Graves' disease) is a rare but serious disorder that is generally transient, occurring in only about 2% of the offspring of mothers with Graves' disease (GD). Cardiac insufficiency and mortality, intrauterine growth retardation, prematurity, craniostenosis, microcephaly and psychomotor disabilities are the major risks in these infants, highlighting the importance of TRAb determination throughout pregnancy in women with GD, and of the early diagnosis and treatment of foetal and neonatal hyperthyroidism. Antithyroid drugs (ATDs) are the treatment of choice for hyperthyroidism during gestation and the neonatal period, but their use during the teratogenic period of early pregnancy may be associated with a higher risk of birth defects and foetal hypothyroidism. Management of the mother, the foetus and the neonate requires an experienced multidisciplinary team including adult and paediatric endocrinologists, obstetricians and foetal radiologists.

Pathogenesis

Autoimmune foetal and neonatal hyperthyroidism is commonly caused by the passage across the placenta of maternal stimulating antibodies directed against the TSH receptor (TRAbs). These antibodies stimulate the adenylate cyclase in foetal thyrocytes, leading to the hypersecretion of thyroid hormone. The prevalence of hyperthyroidism in pregnancy is about 0.2%, with most cases due to Graves' disease. Graves' thyrotoxicosis generally improves in the second half of pregnancy, due to decreases in serum TRAb concentration, but it then worsens after delivery [1]. The preservation of normal foetal thyroid hormone status, which is essential to ensure normal brain development, is a complex issue in cases of maternal gestational autoimmune Graves' disease. High levels of antibody transmission are associated with the occurrence of foetal thyrotoxicosis. Foetal hyperthyroidism may develop when foetal TSH receptors become physiologically responsive to TSH and to TSH receptor antibodies, during the second half of gestation, at around week 20, mostly in women with high levels of TRAbs. It may also occur in the children of mothers who were treated years earlier for Graves' disease but still have circulating TRAbs. Thus, all pregnant women with GD and euthyroid pregnant women with a history of GD (and/or receiving long-term levothyroxine treatment after radioiodine or thyroidectomy related to GD) should

undergo TRAb determinations at the beginning of pregnancy. If TRAbs are detected, the foetus should be considered at risk of developing thyrotoxicosis and monitored accordingly (figure 1) [1, 2].

Autoimmune foetal and neonatal hyperthyroidism must be distinguished from other less frequent mechanisms of non-autoimmune congenital hyperthyroidism [3]. Non-autoimmune neonatal hyperthyroidism, due to an activating mutation of the TSH receptor gene or McCune Albright syndrome (activating mutation of the *Gsα* gene), is a very rare disease. Molecular abnormalities of the TSH receptor, leading to its constitutive activation, may be responsible for severe permanent congenital foetal and postnatal hyperthyroidism. Germline mutations are found in cases of hereditary autosomal dominant hyperthyroidism, and *de novo* mutations may cause sporadic congenital hyperthyroidism. The clinical course of these diseases requires careful management. Even with high doses of ATDs to control severe congenital thyrotoxicosis, thyroid nodules may develop and goitre enlargement may occur early in life, necessitating subtotal thyroidectomy followed by radioiodine therapy. Autonomous adenomas with autonomous thyroid hormone secretion due to somatic mutations of the TSH receptor gene and abnormally high levels of constitutive TSH receptor activity remain exceptional and are much rarer in neonates and children than in adults. Surgical excision of the nodule definitively cures the hyperthyroidism.

This review focuses on the management of autoimmune hyperthyroidism during the foetal and neonatal periods.

Clinical manifestations

Foetal hyperthyroidism is almost invariably followed by neonatal hyperthyroidism. Neonatal autoimmune hyperthyroidism is generally transient, occurring in only about 2% of the offspring of mothers with GD. However, it is associated with a risk of mortality and immediate and long-term morbidity. Foetal and neonatal thyroid function may be disturbed to various extents by the presence of TRAbs, the use of ATDs and maternal thyroid hormone status. If maternal disease is untreated or poorly controlled, goitre, intrauterine growth retardation, oligoamnios, prematurity and foetal death may occur [4]. Tachycardia, hyperexcitability, poor weight gain in children with a normal or large appetite, goitre, staring and/or eyelid retraction and/or exophthalmia, small anterior fontanel, advanced bone age, hepatomegaly and/or splenomegaly are the most frequently observed clinical features during the neonatal period. Cardiac insufficiency is one of the major risks in these infants. Biological abnormalities of the liver may also be observed in the absence of cardiac insufficiency. Craniosynostosis, microcephaly and psychomotor disabilities may occur in severely affected infants [3, 5].

Diagnosis and management

During pregnancy

The early diagnosis and treatment of foetal hyperthyroidism or hypothyroidism are crucial and highlight the importance of TRAb determination throughout pregnancy, in women with Graves' disease. Current guidelines recommend TRAb determination early in pregnancy and during the second half of gestation, starting from 20-24 weeks of gestation, with close monitoring if TRAb levels exceed two to three times the upper limit of the normal range [6]. These at-risk pregnancies should be monitored carefully, with repeated ultrasound examinations of the foetal thyroid gland [7]. The experience of the ultrasound operator is also crucial to the management of pregnancy in women with GD. Foetal thyroid width and circumference should be determined from 20 weeks of gestation onwards [8]. In foetuses with goitre, the main clinical issue is determining whether the cause is maternal ATD treatment that is appropriate for achieving normal maternal thyroid function but inappropriate and excessive for the foetus resulting in hypothyroidism, necessitating a decrease in ATD dose, or whether the cause is foetal thyroid stimulation by maternal Graves' disease, with the presence of TRAbs resulting in foetal thyroid stimulation and hyperthyroidism, requiring an increase in maternal ATD dose (figure 1).

Foetal ultrasound scans are a non-invasive tool for detecting foetal thyroid dysfunction. Such scans should be performed monthly, from 20 weeks of gestation onwards, to screen for goitre and evidence of foetal thyroid dysfunction in pregnant women with GD testing positive for TRAbs and/or receiving ATDs. Thyroid gland enlargement is the starting point for the diagnosis of thyroid dysfunction, and ultrasound scans are also used to assess the presence and vascularity of the goitre. A positive signal at the periphery of the thyroid gland has been shown to be associated with foetal hypothyroidism, whereas a positive signal throughout the gland is linked to foetal hyperthyroidism [7]. Assessments of foetal bone maturation (advanced bone maturation in cases of foetal hyperthyroidism, with a distal femoral centre seen before the normal physiological appearance of this structure at a gestational age of 32 weeks; delayed bone maturation, with no visible distal femoral centre after 32 weeks of gestation in cases of foetal hypothyroidism) and foetal heart rate (greater than 160/min in cases of foetal hyperthyroidism) may also facilitate the diagnosis of hypo- or hyperthyroidism, guiding the choice of the most appropriate treatment (figure 1) [2]. Invasive examinations, such as foetal blood collection or amniotic fluid sampling, are not usually required and should be reserved for rare cases in which the diagnosis is dubious or intra-amniotic thyroxine injection is required to treat a secondary foetal hypothyroid state [2] if hypothyroidism persists two to four weeks after ATD dose reduction, with propylthiouracil

(PTU) \leq 100 mg/d or methimazole/carbimazole (MMI/CMZ) (CMZ is a precursor of MMI) with CMZ \leq 10 mg/d. A combination of maternal criteria (TRAb titres, ATD use and dose) and foetal criteria (thyroid Doppler signal, foetal heart rate and bone maturation) is used to distinguish between foetal hypothyroidism and hyperthyroidism [7].

During the neonatal period

The prenatal response to treatment, based on foetal status and the results of thyroid function tests carried out on cord blood at birth, may validate the prenatal treatment strategy, but is not predictive of subsequent neonatal thyroid dysfunction. Remarkably, only a minority of neonates born to mothers with gestational autoimmune thyroid disease have disturbed thyroid hormone levels [7, 9, 10]. Neonates from mothers testing negative for TRAbs during the second half of gestation (with negative tests on cord blood) can be discharged and require no further follow-up [9, 11]. Hyperthyroidism may develop in neonates within two to five days of birth, if TRAbs persist after the clearance of transplacentally transmitted ATDs from the mother. A threshold value for a maternal second-generation thyroid binding inhibitory immunoglobulin assay of 5 IU/l during the second half of gestation and delivery has been recently identified for defining risk situations for foetal and neonatal hyperthyroidism [12]. A close relationship has been demonstrated between serum maternal TRAb levels at the end of gestation and the levels of these antibodies in the serum of the neonate. If TRAbs are detectable, thyroid function tests should be repeated in the first week of life (every two days), even if normal (or high TSH levels due to excessive ATD treatment in late gestation) results were obtained with cord blood. Most cases of neonatal autoimmune hyperthyroidism are diagnosed within the first two weeks of life [11]. Neonates born to mothers with very low TRAb levels (less than two to three times the upper limit of the normal range; values depending on the assay, but usually below 5 IU/l) may have serum FT4 levels at about the 95th percentile on day 2-5, with these levels subsequently decreasing to within the normal range during the second week of life [10]. A strong suspicion of neonatal autoimmune hyperthyroidism when TRAbs are detectable and present at high levels (more than three times the upper limit of the normal range; generally > 5 IU/l) in cord blood and free thyroid hormone levels are high in the first two to four days after delivery (FT4 levels above the upper limit of the normal range for age, ≥ 40 pmol/l), should lead to the initiation of ATD treatment in the infant shortly after birth, to prevent the development of clinical hyperthyroidism, thereby protecting the infant from the serious consequences of this condition. In rare cases, transient neonatal hypothyroidism may occur for one to two weeks, due to the simultaneous presence of maternally transmitted thyrotropin receptor blocking antibodies (TBAbs), and an imbalance in TSAbs and the TBAbs levels. Neonatal hyperthyroidism then occurs, in which TRAbs predominate, highlighting the need for

repeated measurements of serum thyroid hormone levels during the first two to four weeks of life in some cases, depending on serum TRAb levels. Less widely available third-generation bioassays determining the levels of thyroid-stimulating or blocking immunoglobulins through the monitoring of cyclic adenosine monophosphate production may be used in these cases.

Treatment

During gestation, ATD treatment is commonly used to achieve euthyroidism in women with GD. However, both MMI/CMZ and PTU are associated with an increase in the prevalence of birth defects (including maternal agranulocytosis and liver failure, both of which are usually very rare), but the spectrum of malformations differs between these drugs [13]. Exposure to MMI/CMZ during the teratogenic period of pregnancy (6-10 weeks of gestation, corresponding to weeks 4 to 8 of embryonic development) is associated with a higher risk of choanal atresia, omphalocele, oesophageal atresia, omphalomesenteric duct abnormalities, aplasia cutis congenital, nipple and eye malformations. The birth defects associated with PTU exposure are milder and appear to be restricted to face and neck malformations. Exposure to MMI/CMZ or PTU is also associated with a higher risk of malformations of the urinary system [14]. Such birth defects are observed in about 3% of neonates, and some birth defects may be detected later, resulting in a total prevalence of about 6% at two years of age. It has, therefore, recently been suggested that the use of ATD at 6 to 10 weeks of gestation should be limited as much as possible, to decrease the risk of birth defects [15].

Foetal hyperthyroidism can be prevented by administering antithyroid drugs to the mother. PTU and MMI/CMZ cross the placenta and are equally effective for treating hyperthyroidism in pregnancy. PTU is the most widely used during pregnancy, generally with a shift onto this drug from MMI/CMZ shortly before conception [15]. The foetus benefits directly from the maternal ingestion of these drugs, which cross the placenta and act on the foetal thyroid gland. However, these drugs may also expose the foetus to the risk of hypothyroidism, and small doses (usually no more than 100-150 mg PTU or 10-15 mg MMI/CMZ daily) are, therefore, recommended during the second half of gestation (figure 1).

During the neonatal period (table 1), MMI/CMZ is preferred (1 mg/kg/day, in three divided doses). Propranolol (2 mg/kg/day, in two divided doses) can also be used to control tachycardia during the first one to two weeks of treatment. It is usually possible to decrease ATD dose progressively, according to thyroid hormone levels. Levothyroxine may be added to the regimen, but dose titration should be preferred over “block and replace” approaches.

The disease is transient and may last from one to three months, until maternal TRAbs are eliminated from the infant's bloodstream circulation. Mothers can breastfeed while taking ATDs (usually with a MMI/CMZ dose of less than 20 mg per day, or a PTU dose of less than 300 mg per day), with no adverse effects on the thyroid status of their infants [16].

Outcome

Craniosynostosis is rarely reported but should be diagnosed and managed as early as possible to improve outcome. Transient central hypothyroidism due to thyroid regulatory system impairment as a result of inadequately treated maternal GD is rare [17], but may become apparent after the clearance of ATDs. It requires levothyroxine treatment for several weeks and highlights the need for careful monitoring of thyroid function after the resolution of neonatal hyperthyroidism.

Despite the favourable outcome, follow-up studies are required to evaluate the long-term neuropsychological, emotional and behavioural functioning of children with neonatal hyperthyroidism.

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Figure 1: Management algorithm for at risk pregnancies in mothers with current or past hyperthyroidism, mostly due to Graves' disease

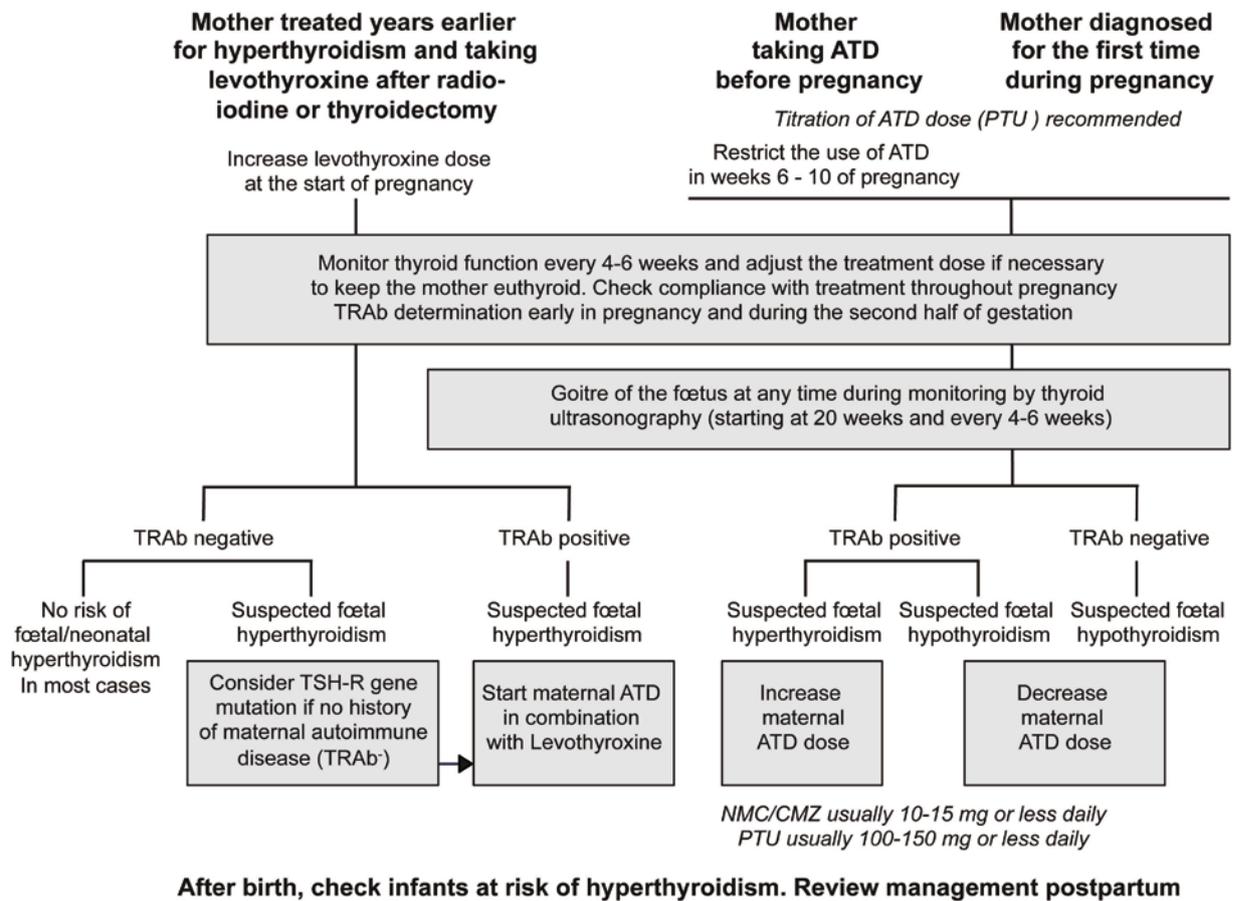


Table I: Management for neonates with autoimmune hyperthyroidism

- Determine TRAb in cord blood: High risk of neonatal hyperthyroidism if TRAb > 5 IU/l; and FT4, TSH levels: may validate the prenatal strategy, but not predictive of subsequent thyroid function
- Repeated measurements of serum thyroid hormone levels during the first 2 weeks of life: days 3, 5, 7, 10, 15
- Physical examination. Check for malformations
- Admission to hospital for the first week of life
- Initiate MMI/CMZ treatment as soon as possible: 1 mg/kg/d divided into 2-3 doses
Dose should be decreased when serum FT4 levels are within the reference range
- Propranolol: 2 mg/kg/d divided into 2 doses, for 2 weeks
- Repeated measurement of serum thyroid hormone levels weekly until stable, and then every 2 weeks
- Dose titration should be preferred, but "block and replace" (levothyroxine) strategies may be considered in some cases
- Safety of breast feeding
- Treatment should be stopped when TRAb is no longer detectable in serum
(1 - 3 months, depending on initial level)
- Outcome: check for craniosynostosis, transient central hypothyroidism, long-term neuropsychological development



Meet the Expert Session
5.1 & 5.2

Hypercholesterolaemia in children

Albert Wiegman (Amsterdam, The Netherlands)

Sunday 11 September 08:00 – 09:00 – 342 AB
Monday 12 September 08:00 – 09:00 – 252 AB

Pediatric FH

'Hundred years ago, a wonderful new drug was discovered. If you put it on your teeth every day, you will still have teeth when you are eighty years old. You probably know that drug: it's called tooth-paste. Twenty-five years ago, a toothpaste for the blood vessels was invented. If you take that statin, it will brush your vessels clean and you will still have clean vessels when you are eighty years old.'

This story is told for 23 years now, to over 3350 children visiting the Paediatric Lipid Clinic at the Academic Medical Center in Amsterdam. We collected scientific evidence behind the above-mentioned story, with the focus on children suffering from familial hypercholesterolemia (FH). This resulted in a fruitful and still on-going line of research; a group-effort of several researchers.

Almost all children have a dramatic family history, with close family members suffering from cardiovascular disease at a very young age. FH is a co-dominant monogenic disorder with a prevalence of 1:250 individuals, therefore the most frequent monogenic disorder in childhood and is characterized by severely elevated levels of LDL-C from birth onwards.

In the study of early atherogenesis, children with familial hypercholesterolemia proved to be an excellent model for the elucidation of important risk factors. We in fact started to study this question in more than 1000 children. To conclude, in FH families, LDL-c levels (>3.5 mmol/L) allow accurate diagnosis of FH in childhood, and moreover, increased LDL-c (>6.23 mmol/L) and lipoprotein(a) (>300 mg/l) and decreased HDL-c levels (<1.0 mmol/L) in children identify FH kindred with the highest CAD risk. (Wiegman e.a. Circulation 2003)

Patients with FH suffer from severe CAD early in life. Should lipid-lowering treatment started in childhood? We therefore assessed 201 children heterozygous for FH and 80 unaffected siblings (both age ranges 8–18 years) with ultrasound to measure carotid wall intima-media thickness. Mean carotid IMT of heterozygotes was significantly greater than that of unaffected siblings. A significant deviation in IMT was noted before age 12 years. LDL-c, age, and sex showed to be strong and independent predictors of IMT. Since raised LDL-c can be lowered efficiently, studies were needed to investigate long-term safety and effectiveness of statin treatment in children with FH. (Wiegman e.a. Lancet 2004) Later on, carotid IMT was measured at baseline of a rosuvastatin trial and there was already a significant difference in thickness between both groups before the age of 8 years. (Kusters e.a. Circ Research 2014)

Treatment of FH patients with cholesterol-lowering medication, preferably from a young age, is mandatory to prevent premature CVD. Statins have been widely accepted as effective drugs to reduce morbidity and mortality in patients with FH and are considered safe for long-term use.

Despite their efficacy, statins were not prescribed to children with FH until about 15 years ago, because evidence regarding the efficacy and safety in children was completely lacking. Carotid intima-media thickness (cIMT), an established surrogate marker of cardiovascular disease, is already increased in young children with FH as compared with their unaffected siblings. Two years of treatment with pravastatin reduces cIMT, and pravastatin is safe in children. In an international, multi-centre trial (Lipid-cohort), it was proven that simvastatin is safe and efficacious as well, albeit in the short term, and that statin therapy has a favourable effect on endothelial function in children with FH. In a 5-year follow-up study of the 'Lipid'-cohort, it was shown that statins in children are safe in the longer term also, and that the age at which statin treatment was initiated, was a significant determinant of cIMT in young adulthood. This implicates that the younger statin therapy is started, the more efficacious the process of atherosclerosis is halted.

Would early intervention with pravastatin inhibit the process? Treating 214 children with FH (aged 8 to 18 years) in a randomized, double-blind, placebo-controlled, two-year trial with pravastatin 20-40 mg, compared to baseline, carotid IMT showed regression on pravastatin and progression in the placebo group. The change of IMT between the two groups differed significantly. No differences were observed for growth or maturation between both groups. So, early intervention with pravastatin is efficacious, safe and therefore probably desirable. (Wiegman e.a. JAMA 2004)

Mean follow-up of the long term pravastatin study was 4.7 years and statin-use proved to be effective and safe. The age at which statins were introduced in children was a significant independent predictor on cIMT. Earlier initiation of statins will prevent premature atherosclerosis. (Rodenburg e.a. Circulation 2007)

Currently, twenty-six children on pravastatin exceed the age their affected parent suffered their first myocardial infarction or died. After ten years of treatment in 213 children, only one child stopped treatment due to side effects. A few stopped during pregnancy, but continued afterwards. The IMT is increasing in the 10 year follow up, but at the same speed as the IMT of their unaffected siblings. (Kusters e.a. JAMA 2014)

Rosuvastatin for children with FH shows to be safe and effective as well. (Braamskamp e.a. J Clin Lipidol 2015) Together with the ten-year follow-up study of the 'Lipid' cohort, this led to a new consensus. (Wiegman e.a. Eur Heart Journal 2015)



Meet the Expert Session
6.1 & 6.2

Prolactinomas in adolescence

Philippe Chanson (Paris, France)

Sunday 11 September 08:00 – 09:00 – Salle Maillot
Monday 12 September 15:15 – 16:15 – 252 AB

Management of prolactinoma in adolescence

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Even if prolactinomas are rare in children and in adolescents, pediatricians must be aware of this potential diagnosis in young patients with growth retardation and, overall with pubertal delay and/or primary amenorrhea. Secondary amenorrhea, galactorrhea may also be presenting symptoms. Diagnosis relies on prolactin (PRL) measurement. If PRL levels are increased, pituitary magnetic resonance imaging (MRI) needs to be performed in order to differentiate between microadenoma (adenoma of less than 10 mm maximal diameter) and macroadenoma (more than 10 mm maximal diameter). This latter can expand laterally (to the cavernous sinus) or upwards, in the direction of optic chiasm that it can compress, leading to visual signs (particularly visual fields defects). Whether micro- or macroadenomas, prolactinomas are treated medically with dopamine agonists (DA). The preferred drug is cabergoline, which is the most effective and tolerable DA. The medical treatment is able not only to normalize PRL levels (and thus to restore gonadal function) in the vast majority of cases but also to produce a rapid shrinkage of the tumor mass, relieving visual problems. Genetic screening for *AIP* and *MEN1* mutations may be proposed.

Epidemiology

The prevalence of pituitary adenomas is much lower in childhood and adolescence than in adulthood. As in adults, most pituitary adenomas are prolactinomas, representing 50% of all pituitary adenomas in this age group (1-4).

A compilation of various series shows that, in this age group, prolactinomas are mainly observed in girls (79%) and that macroprolactinomas are more prevalent than microprolactinomas (58% *versus* 42%) (5-13). This high proportion of macroprolactinomas is clearly very different from what is observed in adults where microprolactinomas predominate (14).

Ocurrence at young age may be related to genetic predisposition

The *AIP* gene has been implicated in pituitary tumorigenesis in families with pituitary adenomas (FIPA), particularly those with GH-secreting adenomas. *AIP* mutations have also been detected in patients with apparently sporadic adenomas: we found that 2.2% of 433 patients in whom an adenoma was diagnosed in adulthood had *AIP* mutations; such mutations were more frequent in children (23%), particularly those with a GH- or mixed GH-PRL-secreting macroadenoma and also, but more rarely, those with prolactinomas (15). Apparently sporadic pituitary adenomas in young patients may also be one of the first manifestations of *MEN1* (16). In our series of young patients with apparently sporadic macroprolactinomas, the prevalence of *AIP* mutations was relatively low (9%) and close to that of *MEN1* mutations (5%).

Whether all young patients with prolactinomas need to be tested for *AIP* and *MEN1* mutation remains debated (16).

Clinical manifestations

Most macroprolactinomas in this age group are diagnosed during adolescence (5-13). In our series of 77 patients, median age was 17 years and only two patients were less than 10 years old at diagnosis (13).

Children and adolescents with a prolactinoma commonly present with pubertal delay in ~ 50% of cases, growth retardation in ~ 20-25%, or primary amenorrhea, in addition to the more classical presentations of galactorrhea, secondary oligo/amenorrhea and mass effects (6,7,10,13).

In girls, the diagnosis is generally suggested by amenorrhoea (80% of cases), which may be primary or more often secondary. Oligomenorrhoea and irregular cycles are less frequent. Breast development usually occurs despite primary amenorrhoea, and complete absence of pubertal development is rare (13). In boys, hypogonadism or gynecomastia may point to the diagnosis but a tumor mass syndrome is generally the primary presenting feature (13). Growth retardation is rare and observed in children with macroadenomas (6).

Prolactinomas appear to develop around puberty, a phase associated with increased sex steroid secretion. It is conceivable that they develop earlier but are only diagnosed because of their effect on puberty (e.g. amenorrhea). The impact on gonadotroph function is more likely due to hyperprolactinemia than to a mass effect on normal pituitary cells, as growth perturbations are not as frequent as pubertal disorders (24% vs 49% in our series) and panhypopituitarism is relatively rare.

Weight gain and obesity were reported in half the patients in two series (10,13). The precise mechanisms of this weight gain remains speculative. It seems to be related to the high levels of PRL as it is also observed in patients with microprolactinomas which have no hypopituitarism or hypothalamic involvement.

Pituitary mass effect is more severe in boys than in girls

The tumors seem to affect puberty or growth more often in boys than in girls (61% vs 43% and 46% vs 14%, respectively in our series) (13). This cannot be explained by age at diagnosis, which was similar in the two genders, but is probably rather related to the size of the adenoma, which was larger in boys, as it is in male adults (10); this would also explain the higher prevalence of visual consequences in boys. It must be also pointed out that many of the youngest patients do not complain of visual disorders, which can delay the diagnosis (5-12); this was the case of the two youngest boys in our series, who had unilateral blindness (13).

In contrast to adult patients, there is a disproportionately larger number of pediatric patients who have macroadenomas (60%) and invasive adenomas (50%), particularly in boys, and the proportion of boys who present with a macroadenoma at diagnosis is greater than in girls (81% vs. 59%) (6,7,10,13). The reasons for the higher percentage of large invasive PRL tumors among children and adolescents are not known, but about 15% exhibit an AIP or MEN1 mutation, independent predictors of dopamine agonist (DA) resistance (13).

Medical therapy is the first-line treatment as DA are very effective.

Medical therapy with DA is the first-line treatment of choice for adults with macroprolactinomas (14). This is also the case for adolescents and children. Indeed, medical therapy is effective not only in normalizing PRL levels but also in reducing tumor mass in children or adolescents with prolactinomas. In our experience (13), DA were used as primary treatment in nearly 90% of young patients with macroprolactinomas, and only a few underwent initial surgery for visual disturbances; response to dopamine agonists (cabergoline in the majority of cases) was very favorable, with PRL normalization observed in more than three-quarters of cases during first-line treatment and one-half of cases when started after surgery. Overall, PRL levels normalized in 73% of young patients and tumor shrinkage was observed in 76% of cases (13). These results confirm that dopamine agonist therapy is as effective at this young age as in adulthood (5-7,9,10,12).

Dopamine agonists act very quickly on tumor mass allowing a rapid shrinkage of the tumor and allowing rapid (few hours/days) improvement of visual defects when the tumor has compressed the optic chiasm. Thus even in the case of optic chiasm compression, surgical intervention should be avoided and medical treatment must be preferred. Reduction of tumor size usually restores pituitary function (13,17).

The DA of choice is cabergoline which is the most potent and tolerable DA. It is generally initiated at the dose of 0.025 mg per week and increased to 0.5 mg per week after 2 or 3 weeks if well tolerated. The dose may be increased after few weeks if the effect on PRL levels and/or tumor volume is not satisfactory.

DA-resistance is very rare.

Very few patients (<10%) do not normalize PRL levels even after increasing the dose of DA. As in adults, DA-resistant patients tend to be younger males with larger adenomas that are more often invasive and display higher proliferation index. *AIP* mutations were not associated with DA resistance in young patients, but *MEN1* mutations, in contrast, may be associated with more aggressive and more resistant macroprolactinomas (13).

In some very rare cases of DA resistance, surgical debulking may be indicated (10,13). Nowadays, as in adults, there is no place for radiotherapy for in children and adolescents with macroprolactinomas.

Long-term follow up

Long-term follow-up of children and adolescents diagnosed and treated for prolactinomas shows no association with major adverse health consequences, aside from those specific to radiotherapy in those very rare children treated with this modality in the past (5,7).

Osteopenia has been observed in young hyperprolactinemic patients. In one study, 22 patients diagnosed with prolactinomas before the age of 18 years had lumbar spine bone mineral density significantly lower than in age-matched controls (18) and treatment with a DA improved, but did not normalize, bone mineral density values (18). Whether reduced bone mass associated with hyperprolactinemia translates into an increased risk for future osteoporotic fractures is not known.

In adults there has been concern about valve disease occurrence under prolonged DA treatment, particularly in patients with Parkinson's disease. In fact this has not been confirmed in prolactinoma patients or, if it exists, it seems limited to patients treated with very high doses and for very long periods of time (19). There are no available data on cardiac valve status in this age range but regular clinical examination looking for a cardiac murmur (more than systematic echocardiography) is recommended.

Conclusion.

Prolactinomas are rare before the age of 20 years. They generally affect girls, occur during adolescence, and are very rare in young children. They are as sensitive to DA therapy as prolactinomas in adults, making this the first-choice treatment for this young population. As in adult patients, DA resistance is associated with higher PRL levels and larger tumors, the latter two parameters being linked, as expected with regard to adult patients with prolactinoma. *AIP* mutations are less frequent than in young patients with acromegaly or gigantism. *MEN1* mutations are not rare in these patients and seem to be an independent predictor of DA resistance. More studies are needed before recommending routine *MEN1* screening in young patients with isolated, non familial prolactinomas.

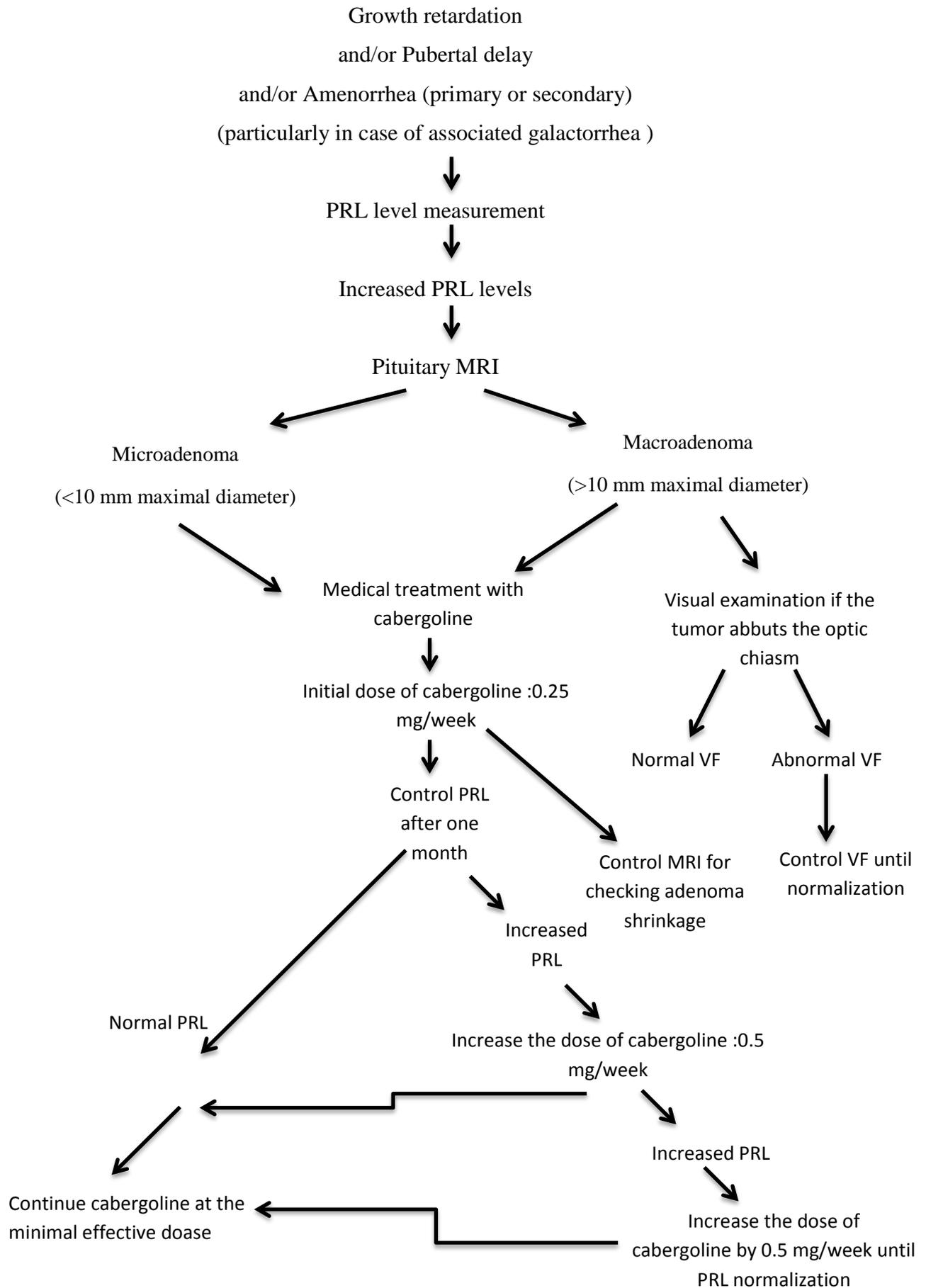
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Practice flow diagram for diagnosis and treatment





Meet the Expert Session
7.1 & 7.2

Neonatal hypocalcaemia

Serap Turan (Marmara, Turkey)

Sunday 11 September 08:00 – 09:00 – Amphithéâtre Bordeaux
Monday 12 September 15:15 – 16:15 – Salle Maillot

Neonatal Hypocalcemia

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Key words

Hypocalcemia, hypoparathyroidism, neonate, hypoparathyroidism

Introduction

The first 28 days of life is a unique period of life for the etiology and clinical manifestations of hypocalcemia. Both maternal and fetal factors affect the calcium and mineral homeostasis and environmental factors such as nutrition easily cause pathologies in newborns.

Calcium transfer from mother to fetus occurs via active transport under the control of parathyroid hormone-related peptide and, resulted in relative fetal hypercalcemia, especially in last trimester. At birth, with abrupt cessation of this transport, serum calcium concentration falls in newborn. In the 1st weeks of life, newborns are relatively hypocalcemic compared to adults (1). For that reason, preterm babies who have ex-utero life in the third trimester are under risk of low total body calcium and hypocalcemia.

Definition Hypocalcemia is defined as a serum total calcium (tCa) level of <2 mmol/L (8 mg/dL) in normal birth weight (BW) newborns and <1.75 mmol/L (7 mg/dL) in very low birth weight (VLBW) newborns (BW <1500 g). Ionized Ca (iCa) levels for hypocalcemia definition are <1.1 mmol/L (4.4 mg/dL) and <0.9 mmol/L (3.6 mg/dL) in normal and VLBW newborns, respectively (2).

Calcium binds to proteins, mainly to albumin, in plasma and, total serum Ca level is the sum of iCa and bound Ca. In case of low protein levels, i.e. hypoalbuminemia, tCa level is low, however, the iCa level is normal. In this condition, Total Ca levels can be corrected according to serum albumin level by using following formula;

$$\text{Corrected Ca "mg/dl"} = \text{tCa "mg/dl"} + [0.8 \times (4 - \text{albumin "mg/dl"})]$$

Acid-base status also affects iCa level. Binding of Ca to albumin reduces in acidosis; while increases in alkalosis. Thus, acute respiratory alkalosis can induce signs of hypocalcemia by decreasing iCa; while metabolic acidosis can conceal hypocalcemia symptoms by increasing iCa. Correction according to serum pH could be performed in a principle of 0.1 unit change in pH causing the 0.16 mg/dL (0.04 mmol/L or 0.08 mEq/L) approximate change in iCa at reverse direction (3).

The measurement of iCa concentration is more practical and reliable than the serum tCa levels, particularly in newborns that are premature or ill, due to high prevalence of hypoproteinemia and disturbances in acid-base status in these babies (1).

Specific symptoms of hypocalcemia are tetany, laryngospasm and seizures. Hyperexcitability, irritability, jitteriness and facial spasms are specific but more subtle symptoms (2, 4). The clinical signs of hypocalcemia in neonatal period may be nonspecific such as apnea, tachycardia, cyanosis, emesis, and feeding problems (2).

Etiology

Neonatal hypocalcemia is classically classified as “early” and “late” depending on the postnatal day of presentation, etiology changes accordingly. When hypocalcemia occurs in the first 2-3 days after birth, it is considered to be early. This classification generally makes diagnostic approach more simplistic. In most cases, the diagnosis can be established with an initial laboratory results, supported with a detailed history and physical examination. However, in some conditions, early hypocalcemia could be mild and asymptomatic and, can be diagnosed after 72 hours. On the other hand, although in first 72 hours of life hypocalcemia is mostly due to fetal and maternal conditions or neonatal systemic disease like asphyxia and sepsis, sometimes, late hypocalcemia could be related to the these conditions. Thus, the clinicians should always be careful while categorizing the condition, especially patients presenting at postnatal age of 2-6 days, called as grey-zone.

Early Neonatal Hypocalcemia is an exaggeration of the normal decline in Ca concentration after birth (1). This is mostly a problem of prematurity, intrauterin growth restriction (IUGR), LBW, asphyxia, sepsis and infants born to diabetic mother (4). Different mechanisms have role in development of hypocalcemia during this period, such as, decreased transfer of Ca across the placenta, impaired secretion of PTH, inadequate response to PTH, hypercalcitonemia, hypomagnesemia and relative calcitriol resistance. Also, severe maternal vitamin D–deficiency is a risk for early hypocalcemia.

Approximately one-third of premature infants, the majority of VLBW infants and one third of the infants with birth asphyxia have early hypocalcemia (1, 2). In asphyxiated newborns, seizures are often the result of hypoxic-ischemic encephalopathy, however, could be related to hypocalcemia (5). Increased phosphate load caused by cellular injury, reduced Ca intake, and hypercalcitonemia are important factors for hypocalcemia in asphyxia.

Infants of diabetic mothers are also under risk of early hypocalcemia. Good glycemic control in diabetic mothers reduces the risk (2) and in different series, frequency of hypocalcemia changes from 10% to 50% (1) The proposed mechanism of neonatal hypocalcemia related to the maternal diabetes are decreased placental transfer of Ca due to increased urinary losses of Ca and magnesium in mother, insufficient neonatal PTH secretion, hypercalcitonemia, hypomagnesemia which is detected in 1/2 of the infants of diabetic mother, and nutritional deficiency and/or impaired absorption of ingested calcium (2).

Additionally, in case of maternal hyperparathyroidism, fetal hypercalcemia due to increased placental transfer of calcium, suppresses fetal PTH secretion. Inappropriate response of PTH to low serum Ca may persist for several months, and, abrupt cessation of placental Ca at delivery leads to hypocalcemia with biochemical features of hypoparathyroidism (2).

Late Neonatal Hypocalcemia develops after the third day of life and mostly at the end of the first week (6) and, vitamin D deficiency and hypoparathyroidism are the two main etiological causes. Hyperphosphatemia is another reason of hypocalcemia, which could be related to ingestion of bovine milk or high phosphorus formulas, renal failure and rarely phosphate enemas. Hyperphosphatemia and hypocalcemia may initially suggest hypoparathyroidism, but serum PTH concentrations are high in infants with excessive phosphate loading in response to a reciprocal reduction in serum Ca values (2). Hypomagnesemia are the other common reason of hypocalcemia and hypoparathyroidism. Malignant osteopetrosis type II is a rare cause of late hypocalcemia (1).

Transient late hypocalcemia occurs in many other situations such as therapeutic interventions in ill or premature infants (bicarbonate infusion, lipid infusions, phototherapy for hyperbilirubinemia, exchange transfusion with citrate used store blood), severe rotavirus infection, aminoglycoside antibiotics (e.g. gentamycin).

Maternal vitamin D deficiency is under-recognized reason of hypocalcemia but it may be the one of the most common reason of late neonatal hypocalcemia in some regions (7) Limited maternal sun exposure due to wearing concealing clothes, born in the late winter-early spring or overuse of sunscreens, closely spaced pregnancies and inadequate intake of vitamin D due socioeconomic reasons are the main reasons (4, 7). Adequate maternal vitamin D intake is very important, i.e. 600 IU/day as RDA (9)

Hypoparathyroidism in infancy is usually transient, which is related to the delayed developmental and maturation of parathyroid gland and, frequently resolves within the first several weeks of life. If it prolongs, the error in the embryogenesis of the parathyroid glands or in the synthesis or secretion of parathyroid hormone (PTH) should be considered (2).

DiGeorge syndrome (DGS) a neurocristopathy with a frequency of 1:4000 is the most common form of dysgenesis of the parathyroid glands in neonates. The DGS results from maldevelopment of tissues derived from the third and fourth pharyngeal pouches and first to fifth branchial arches and, approximately 80% to 90% of cases are associated with microdeletions of chromosome region 22q11.2 (del22q11.2: DGCR). DGS is a triad of hypoparathyroidism, immune deficiency (Thymic anomalies-impaired T-lymphocyte and cell-mediated immunity) and congenital heart disease (Conotruncal heart anomalies). Hypoparathyroidism often manifest in the neonatal period, but may not be detected till the adolescence. Velopharyngeal insufficiency, cleft palate, and developmental delay are the other common features of the syndrome. DGS may present in approximately 70% of children with hypoparathyroidism (9, 10). The DGS phenotype without 22q11 microdeletion is associated with microdeletions of chromosomes 10p13 (DGS2), 18q21 and 4q21. The DGS

should be suspected when fetal ultrasonography reveals conotruncal heart disease and confirmed by microarray or fluorescent in situ hybridization (FISH) on samples of chorionic villi or amniotic fluid (2). These studies also should be done in all newborn with permanent hypoparathyroidism with/without other features of DGS. Furthermore, mutations in *TBX1* gene at DGS locus of 22q11.2, also cause DGS.

Several other rare syndromes exhibit multisystem involvement and hypoparathyroidism. The Barakat or HDR syndrome of hypoparathyroidism due to dysgenetic parathyroid glands, sensorineural deafness, and renal failure due to dysplasia or steroid-resistant nephrotic syndrome is associated with heterozygous *GATA3* mutations.

Homozygous mutations in the gene encoding *TBCE* is associated with two different entity, Sanjad-Sakati syndrome characterized by hypoparathyroidism, mental retardation, dysmorphism and Kenny-Caffey syndrome type 1 (KCS1) characterized by hypocalcemia, cortical thickening, medullary stenosis, dysmorphic face, and growth retardation. Kenny-Caffey syndrome type 2 has similar phenotype to type 1, but associated with heterozygous loss-of-function mutations in *FAM111A* (2)

Mitochondrial diseases are also associated with hypoparathyroidism such as Kearns-Sayre syndrome, MELAS and Pearson syndrome (2).

Familial isolated congenital hypoparathyroidism is a rare form of hypoparathyroidism, which is related to more than one genetic defect. Mutations in *GCM2* gene, encoding a DNA-binding transcription factor in parathyroid gland lead to autosomal recessive/dominant form of congenital hypoparathyroidism (2). Inactivating mutations in *PTH* gene interfere the processing of preproPTH to active PTH and, result in functional hypoparathyroidism and transmitted in an autosomal dominant or recessive way. Depending on the specificity of the immunoassay methods used for PTH measurement, serum PTH levels could be low, normal, or even high in these patients (11). X-linked hypoparathyroidism is associated with agenesis of the parathyroid glands; the disorder has been mapped to Xq26-q27 and may involve a deletion-insertion mutation that adversely affects the position of *SOX3* (2). Hypercalciuric hypocalcemia is an autosomal dominant form of hypoparathyroidism due to gain-of-function mutations in *CASR*, which may present in the newborn period. Reduced Ca set point in parathyroid gland and in renal tubular cells results inappropriately low PTH secretion and decreased urinary reabsorption of Ca (2).

Neonatal hypocalcemia due to PTH resistance is a rare condition. Patients having genetic or epigenetic changes causing loss of functional state at PTH receptor or stimulatory alpha subunit of G-proteins (G_{α}) lead to hypocalcemia and hyperphosphatemia with elevated

PTH levels. Loss-of-function mutations in *PTH1R* cause three different autosomal recessive entities as Blomstrand chondrodysplasia type I, type II and Eiken skeletal dysplasia. Loss-of-function mutations or epigenetic aberrations of *GNAS* leading to Gs α deficiency result in pseudohypoparathyroidism (PHP). Patients might also show multihormone resistance i.e. TSH, gonadotropins, GHRH, calcitonin. PHP could be suspected in hypocalcemic infants with hyperthyrotropinemia. Albright's hereditary osteodystrophy (AHO) characterized by short stature, brachydactyly, round face, and heterotopic subcutaneous ossification could be detected in older ages (12). Almost always, only the maternal transmission of the genetic defects causes the hormonal resistance due to imprinting. Recently, EuroPHP-network proposed the term of "inactivating PTH/PTHrP signalling disorder" (iPPSD), which encompass all disorders related to this pathway (13).

Evaluation

Monitorization protocols of newborns under the risk of hypocalcemia should be established, since, most infants with hypocalcemia are asymptomatic. And, iCa is best way of screening preterms, newborns with LBW or asphyxia in NICU, because accurate and fast assessment can be done easily with capillary whole blood if the analyser is calibrated routinely. Total serum Ca should be also measured to confirm the hypocalcemia. Calcium levels in infants with LBW with/without prematurity under 1500 g or hospitalized for any reasons after birth (asphyxia, diabetic mother, sepsis etc.) should be measured in the first 12 hours of life and the measurement can be repeated in first 2 days at least once even the first measurement is normal, since, symptoms of hypocalcemia subtle. Measurement of Ca is not recommended routinely in infants of diabetic mother or late preterm infants if they are not hospitalized and symptomatic. In case of symptoms or any suspicion of hypocalcemia, serum tCa and/or iCa levels should be measured immediately. Clinical manifestations of hypocalcemia are so subtle and can be indistinguishable from other common neonatal diseases (1, 14).

Electrocardiographic evaluation can be performed in infants with hypocalcemia. Prolongation of QT interval corrected for heart rate does not correlate with blood iCa levels (1) but the detection of long QT may be important in monitoring of Ca therapy.

Evaluation for specific etiology is required in infants with persistent early hypocalcemia, late hypocalcemia or hypocalcemic convulsions. Maternal, gestational, peripartum, postnatal, and family history of the newborn should be reviewed for any clue. The physical examination is not diagnostic in most of the cases. Besides the dysmorphic features and cardiac murmurs or cyanosis for DGS, only few physical signs are suggestive for specific etiology, such as alopecia for vitamin D resistance and brachymetacarpals for iPPSD. Serum levels of BUN, creatinine, sodium, potassium, albumin, magnesium and phosphorus, PTH and 25-OH

vitamin D, urinary Ca concentration (spot urine Ca/creatinine ratio) and blood pH, bicarbonate should be measured. If possible, simultaneous serum samples for Ca, phosphorus and PTH should be taken before intravenous Ca administered in symptomatic newborns. In all patients with low or inappropriately low PTH levels, FISH analysis for DGS and echocardiographic evaluation should be performed to detect possible DGS. Serum levels of Ca, phosphate, and PTH should be measured in the mothers of newborn with unexplained hypocalcemia.

Management

Symptomatic hypocalcemia should be treated with 10% calcium gluconate (1 mL/kg) via intravenous route (1). The solution is infused over 5-10 minutes while the heart rate and infusion site are under monitorization. The dose can be repeated in 10 minutes if symptoms continue. Calcium chloride (20 mg/kg or 0.2 mL/kg) is an alternative treatment and this preparation is metabolized more rapidly and can be preferable if it is readily available (1) Prolonged use of Ca chloride in high doses may be associated with hyperchloremic metabolic acidosis and should be avoided (14) Continuous infusion (50-80 mg/kg/day of elemental Ca) probably is more efficacious than intermittent therapy. Mixing Ca solution with bicarbonate or phosphate solution will result in precipitation and should be avoided. (14).

If enteral feedings are tolerated after the initial intravenous Ca therapy, oral Ca therapy containing 75 mg/kg/day of elemental Ca should be started in 4-6 divided doses (i.e. Ca glubionate, Ca gluconate or Ca carbonate)

The long-term effects of PTH therapy are unknown. And, more studies are needed to determine effectiveness and safety of therapy in neonates. In literature, successful recombinant PTH treatment was reported in one neonate with hypoparathyroidism associated with *CaSR* mutation and refractory to conventional therapy (15).

Depending on the etiology of the hypocalcemia, vitamin D supplementation and/or calcitriol treatment are needed. In a neonate with hypocalcemia due to hypoparathyroidism, calcitriol in a dose of 20-60 ng/kg/day and calcium are necessary for restoration and maintenance of eucalcemia. In hypocalcemia due to vitamin D deficiency, 2000 U/day Vitamin D for 90-days are recommended.

For severe persistent hypocalcemia, vitamin D or its analogues is often used in addition to Ca supplementation. Calcitriol (1,25(OH)₂ vit D) is preferred because it can raise serum Ca within 1-2 days after initiation of therapy and leaves no prolong effects within several days of its discontinuation. Vitamin D has slower onset of action of 2-4 weeks and the residual effect

also lasts several weeks after its discontinuation, thus making dosage adjustment more difficult.

Successful management of neonatal hypocalcemia also depends on the resolution of the primary cause of hypocalcemia.

Hypomagnesemia must be treated to obtain maximal response to Ca therapy. Magnesium replacement should be done acutely with the intravenous infusion (15% magnesium sulphate, 1 mL contains 150 mg Mg^{++} , 25-50 mg/kg per dose) over 1-2 hours under cardiac monitorization or by the intramuscular injection of (50% magnesium sulphate, 0.1 to 0.2 mL/kg per dose) twice a day, until the serum magnesium concentration raising over 1.5 mg/dL (0.62 mmol/L). The magnesium concentration should be measured before each dose. One or two doses is usually enough to achieve normal levels. It is necessary to remind that rapid intravenous infusions can cause arrhythmias.

In phosphate-induced hypocalcemia, high-phosphate formulas should be discontinued, Human milk is best choice if possible or low-phosphate formula should be used. Oral Ca supplements should be provided.

Early feeding is the best way to prevent neonatal hypocalcemia, particularly for the preterm infant (14). Early milk feeding and the use of Ca-containing parenteral nutrition (10% calcium gluconate, 50 mg/kg/day) within hours after birth are needed to minimize the development of hypocalcemia. If parenteral Ca infusion is continued for more than 48 hours, additional phosphorus also must be provided based on serum phosphorus measurements (1). Supplementation of Ca decreases the metabolic stress and minimizes the potential for depletion of tissue Ca stores. Calcium (elemental) supplementation doses are to prevent hypocalcemia are 70–80 mg/kg/day for oral or 35-40 mg/kg/day for intravenous. Similar amount of Ca can be provided by an intake of 150 to 200 mL/kg/day of standard term infant formula or human milk.

Complications and Follow-up

Therapy-related complications such as cardiac arrhythmia can be avoided by continuous electrocardiography monitoring during Ca infusion, decrease in heart rate during the infusion is an indication to slow or stop the infusion. Administration of Ca via an umbilical venous catheter, the tip should be in the inferior vena cava, not intracardiac and, direct infusion into the heart might cause arrhythmia. Arterial infusion of Ca in high concentrations should also be avoided, since, administration of bolus dose of Ca via umbilical artery catheter theoretically can lead to intestinal or hepatic necrosis. In peripheral route, the extravasation of Ca solution results tissue necrosis. For these reasons intravenous Ca therapy should be rapidly weaned, or replaced with Ca-containing parenteral nutrition if the infant do not

tolerate enteral feeding. Parenteral nutrition containing standard Ca content can be safely infused through appropriately positioned umbilical venous or arterial catheters (1, 14).

In the presence of hyperphosphatemia, the risk for metastatic calcification is high if Ca treatment is aggressive. In hypoparathyroidism also, there is risk of renal stones, nephrocalcinosis, and possible renal damage.

Long-term outcome of newborn with hypocalcemia depends on the underlying cause. Patients with DGS frequently have neurodevelopmental delay, which is unrelated to hypocalcemia (14). Isolated transient hypocalcemia even in symptomatic cases have not been associated with long-term sequel. However, prolonged seizures might cause neurodevelopment delay. Regular clinical follow-up and laboratory monitoring for Ca, phosphorus and PTH, are necessary even in infants with transient hypoparathyroidism due to the recurrence risk in older ages (14).

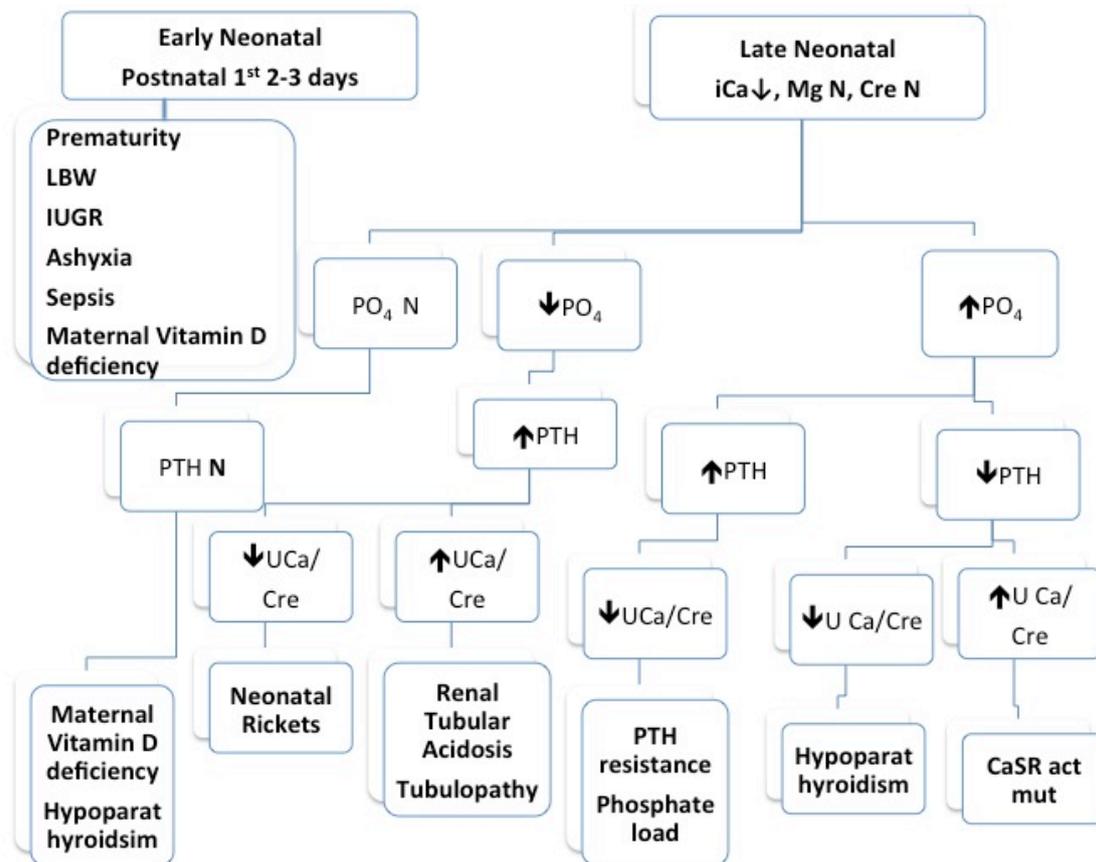
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Figure Legends

Figure: Workings diagram for neonatal hypocalcemia





Meet the Expert Session
8.1 & 8.2

Interpretation of steroid
measurements in paediatric
endocrinology

Yves Morel (Lyon, France)

Sunday 11 September 08:00 – 09:00 – 252 AB
Monday 12 September 15:15 – 16:15 – 342 AB

Steroid measurements in pediatric endocrinology

Yves MOREL, Ingrid PLOTTON, Florence ROUCHER, Véronique TARDY, Faiza CABET and Véronique RAVEROT.

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Steroid measurements are essential in pediatric endocrinology for the diagnosis and clinical investigation of normal physiology and pathologies. Thus, steroid hormones regulate various processes including salt balance, sexual development, reproductive function and immune and stress responses. The pediatric endocrinologist for an accurate interpretation of steroid values should know the biologically influencing factors like age, sex, pubertal stage and circadian secretion. Nevertheless with the recent abundant publications about the steroid measurement by LC MSMS, the pediatric endocrinologists are confusing because they have not access to this new technology and become suspicious to their steroid measurements using immunoassays. The purpose of the “meet the expert” is to debate about the advantages and the limitation of Mass spectrometry and Immunoassays methods and to give to pediatric endocrinologist the ability to understand the limitations of the available IAs and the new possibility to use LC MS MS (see recent review (1)).

1 - Immunoassays

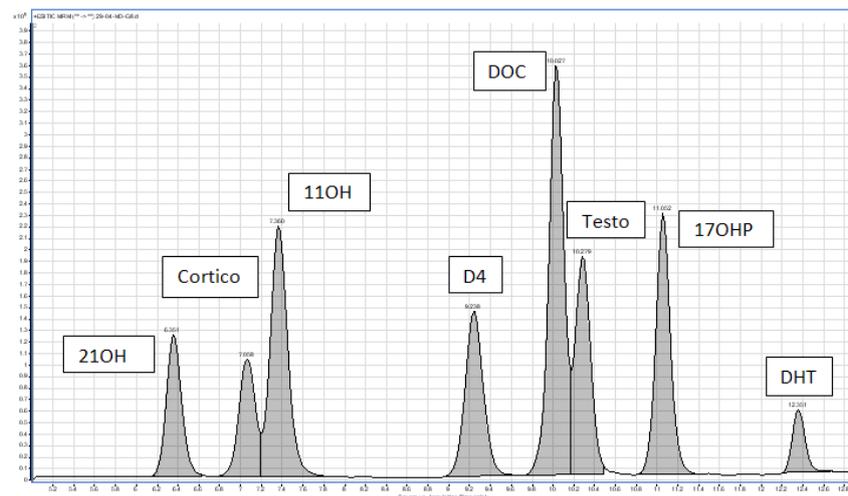
Steroids are small molecules, normally non-immunogenic, but need to be coupled to hapten to be immunogenic. The development of these methods using polyclonal antibodies started around the sixties. First it is a competitive method using radioactive steroid labeled with tritium. To obtain a good sensitivity and specificity, an extraction and a separation by chromatography is necessary. However, as these techniques are time-consuming and laborious, direct immunoassays have been developed without extraction and chromatography. In these two last decades, some direct immunoassays (cortisol, testosterone, progesterone, DHEAS, ..) have moved on automated analyzers allowing rapid results at large scale. They could be done in non-specialist laboratories and do not need special knowledge of personnel. Nevertheless, these methods lack in sensitivity and specificity due to interference with other steroids and plasma steroid binding proteins, matrix effects. Moreover, all commercial steroid kits give a large and problematic variability in results during the inter-laboratory external quality assessment.

In contrast, the steroid values using immunoassays done with pre-analytic treatment (extraction + chromatography to separate the steroids) in a specialized laboratory were identical to these done by LC-MS/MS. However immunoassays allow the measurement of

only a single steroid at a time, remain long and time-consuming and are not adapted to rapid determination in an individual. Actually mass spectrometry based analytical platforms for the determination of single steroid or steroid patterns seems to be an alternative analytical approach with a great specificity.

2- Mass spectrometry

Mass spectrometry (MS) of steroids has been used especially in urine since more of 50 years in steroid research and by some research groups. Gas chromatography-MS (GC/MS) has been first developed around 1960 (see review (2)), is widely used in steroid chemistry and is the gold standard for urinary steroid profiling. Recently several pediatric group have defined normal and pathological steroid metabolomes in urine using GS/MS (3-5). Nevertheless the approach remains long and the interpretation complex. Liquid chromatography tandem mass spectrometry (LC/MS/MS) is replacing all classical methods for steroid hormone analysis. It requires small sample volumes and has given rise to improved specificity and short analysis times. LC permits separation of steroids that are retained on the column stationary phase at differing degrees based on the polarity. Each steroid is ionized and selected in the first quadrupole after which molecular ion fragments, selectively formed in a collision cell, are selected using the second quadrupole. Moreover LC-MS/MS has the advantage that a wide spectrum of steroid hormones can be measured simultaneously. Nevertheless, the plasma sample preparation before LC-MS/MS is critical for routine use. Validation of a LC-MS/MS method should be done for each steroid and requires investigation into a number of parameters such as selectivity, sensitivity, stability especially steroid labeled by deuterium, reproducibility and matrix effects. LC-MS/MS has the advantage that a wide spectrum of steroid hormones can be measured simultaneously as shown on this panel steroid of our laboratory



3 - Some examples

As the advantage of LC-MS/MS is the power to simultaneously measure several different steroids using small volume (200-500 μ l) and with a great specificity, this method is ideal when a lot of steroids and metabolites could interfere in immunoassay and the steroid pattern including ratios is essential for the diagnosis (6). Three applications are adequate:

defects of the biosynthesis of steroids, neonatal period (7) and determination of steroids in amniotic liquid during pregnancy (8, 9).

Another example is the control of elevated 17OHP after neonatal screening of 21-hydroxylase deficiency. Often high value did not match with the diagnosis (absence of virilisation in the female, ..) due to cross-reacting steroids in this neonatal period especially in premature newborns or elevated 17OH-pregnenolone due to HSD3B2 diagnosis. The LC-MS/MS steroid panel could confirm immediately the diagnosis of 21-hydroxylase or HSD3B2 deficiency or eliminated it. In this last case, the child could be discharged from hospital. Already some groups have shift to LC-MS/MS method for neonatal screening (10, 11).

At birth, measurements of testosterone and AMH were often done in DSD patients in emergency using automate or radioimmunoassay without extraction. In these cases, testosterone concentrations were much higher than these obtained by LC-MS/MS or immunoassay after extraction and chromatography. The falsely high results should be at least due to interference of DHEAS that remains very high during the first days of life due to the persistence of the fetal zona. These data confirm the data deduced from UK NEQAS (12).

Conclusion

In the future, LC-MS/MS should be the current method to analysis steroids. However, immunoassays could be not rapidly replaced in routine laboratory. The further development of automatized, more efficient especially in term of sensitivity of LC-MS/MS materials, and more convenient for routine clinical laboratory could progressively give access to steroid measurements by LC-MS/MS. Nevertheless to-day, direct immunoassays provide by common automates will remain in clinical diagnosis. As recently mentioned in the instructions to authors by the endocrine Society after their controversial recommendations requiring MS sex steroid assay, steroid hormone assay measurements require a minimal analytical validity including standards accuracy, precision, specificity, sensitivity, reproducibility and stability (13). Moreover the Endocrine Society “engages industry, governmental or stakeholders to support optimization of all hormone assay measurements to common standards”. The pediatric endocrinologists for a better understanding of the selection and interpretation of steroid determinations should not only take in account of the physiology and pathology of their patient, but also the quality and the limit of the assay.

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