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# Methods of hair loss evaluation in patients with endocrine disorders

## Metody diagnostyki łysienia u pacjentów z endokrynopatiami

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**Abstract**

Hair loss may accompany several endocrine disorders, including hypopituitarism, hypothyreosis, hyperthyreosis, hypoparathyroidism, diabetes mellitus, growth hormone deficiency, hyperprolactinaemia, polycystic ovary syndrome, SAHA syndrome, congenital adrenal hyperplasia, Cushing syndrome, or virilising tumours. Most patients with endocrine disorders present with diffuse non-scarring alopecia, such as anagen effluvium, telogen effluvium or androgenetic alopecia. Focal non-scarring alopecia, such as alopecia areata coexisting with autoimmune thyroiditis, is less frequent and scarring alopecia is a rare finding in patients with endocrine abnormalities. In some cases an endocrine disorder may be suspected based on dermatological findings during hair loss evaluation. Classic methods of hair evaluation include hair weighing, pull test, wash test, the trichogram, and histopathological examination. Newly developed non-invasive diagnostic techniques include the phototrichogram, trichoscan, trichoscopy, and reflectance confocal microscopy.
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***Key words:*** *alopecia, telogen effluvium, androgenetic alopecia, trichogram, trichoscopy*

**Streszczenie**

Łysienie może towarzyszyć wielu chorobom endokrynnym (lub gruczołów dokrewnych) między innymi niedoczynności przysadki, niedoczynności i nadczynności tarczycy, niedoczynności przytarczyc, cukrzycy, niedoborowi hormonu wzrostu, hiperprolaktynemii, zespołowi policystycznych jajników, zespołowi SAHA, zespołowi Cushinga, wrodzonemu przerostowi nadnerczy i guzom wirylizującym. U większości pacjentów z endokrynopatiami obserwuje się rozlane łysienie niebliznowaciejące: anangenowe, telogenowe lub łysienie androgenowe. Rzadziej spotyka się ogniskowe, niebliznowaciejące łysienie plackowate występujące u pacjentów z zapaleniami tarczycy oraz łysienie bliznowaciejące. W niektórych przypadkach wyniki badania dermatologicznego pacjentów z łysieniem mogą nasunąć podejrzenie choroby endokrynologicznej. Klasycznymi metodami badania chorób skóry owłosionej głowy jest ważenie włosów, test pociągania, test mycia, trichogram i badanie histopatologiczne. Nowymi, nieinwazyjnymi metodami diagnostycznymi są: fototrichogram, trichoskan, trichoskopia i obrazowanie włosów i skóry owłosionej głowy metodą refleksyjnej konfokalnej mikroskopii skaningowej *in vivo*.
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***Słowa kluczowe:*** *łysienie, łysienie telogenowe, łysienie androgenowe, trichogram, trichoskopia*

## Introduction

Hair loss may accompany several endocrine disorders, including hypopituitarism, hypothyreosis, hyperthyreosis, hypoparathyroidism, diabetes mellitus, growth hormone deficiency, hyperprolactinaemia, polycystic ovary syndrome, SAHA syndrome, congenital adrenal hyperplasia, Cushing syndrome, or virilising tumours [1, 2]. In some cases, an endocrine disorder may be suspected based on hair and scalp evaluation.

Androgenic alopecia (AGA) is a common disorder affecting both men and women. Although this is an androgen-dependent disease, it most commonly affects persons with normal serum levels of androgens [3]. The pathogenesis of androgenic alopecia is known in more detail in male androgenic alopecia as compared to female androgenic alopecia. In males, the increased expression of 5 $\alpha$-reductase and dihydrotestosterone (DHT) in the perifollicular area drives progressive hair follicle miniaturization and symptoms of hair loss [4]. The role of DHT in female androgenic alopecia is not clear. Some authors (Olsen), indicate that the pathogenesis of hair loss in "androgen-dependent" areas in females is multifactoral and, thus, the term "female pat-

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tern hair loss" should be used for decreased hair density in the central scalp, especially in females who show no abnormalities in serum concentrations of androgens.

Telogen effluvium is hair loss that results from increased premature entering of hair into the telogen phase and, in consequence, increased shedding of telogen hairs. This is an acute or chronic diffuse hair loss which may be caused by a variety of triggers. The most common endocrine triggers of telogen effluvium include hypothyreosis, hyperthyreosis, hyperprolactinaemia, and polycystic ovary syndrome [1, 2, 5, 6].

Alopecia areata is characterized most commonly by acute focal hair loss, which may progress to alopecia totalis. Variants with diffuse or ophiatic hair loss and slow disease progression are not uncommon. About 25% of patients with alopecia areata demonstrate features of autoimmune thyroiditis [7]

Classic methods of hair evaluation include hair weighing, pull test, wash test, the trichogram, and histopathological examination. Newly developed non-invasive diagnostic techniques include the phototrichogram, trichoscan, trichoscopy, and reflectance confocal microscopy [3]. This review summarizes current hair and scalp diagnostic and monitoring techniques which may be useful in evaluating and monitoring hair loss in patients with endocrine disorders.

## Wash test

The wash test is an uncomplicated method, developed to distinguish between androgenic alopecia and telogen effluvium. The method was based on the notion that in telogen effluvium hairs will be shed during hair washing in increased numbers, while in androgenic alopecia there would be no active shedding. Despite an attempt to reactivate this method in recent years, it should be considered a historical and inadequate method in modern dermatology as it lacks specificity and sensitivity [8].

## Hair pull test

The hair pull test is a simple, in office test to estimate the activity of hair loss. In a patient who did not wash their hair for over 24 hours before examination, about 40–60 hairs are grasped between the index finger, middle finger, and the thumb. The hairs are then pulled gently but with firm pressure as fingers slide along the hair shaft. The test is positive, when 6 or more hairs remain in the hands of the examiner [9]. This procedure may be repeated in three scalp areas: frontal, occipital, right or left parietal. Hairs which remain in the fingers of the examiner are telogen hairs, and their number corresponds to the percentage of telogen hairs on the scalp. Test result is positive in telogen effluvium, but is not specific for this disease. It can also be positive in the active phase of alopecia areata or anagen alopecia. The test is difficult to perform in patients with short hair. The pull test is a very approximate method, difficult to standardize, with low specificity and sensitivity, but it might be helpful as a secondary procedure to assess activity of hair loss.

## Hair weighing

Hair weighing is a diagnostic tool for hair growth evaluation in clinical studies. It is used to analyze the effect of topically or systematically applied drugs or cosmetic molecules. A 1.34 $cm^2$ area in the fronto-partial region is shaved. The site is permanently marked by a tattoo. After a treatment-free period of 4–24 months, hairs are clipped and collected. The area is then shaved again. The therapeutic agent is applied and hair is allowed to grow for the same time period as in the previous phase of the study. Again, hairs are clipped and collected. Then the hair collected from both sampling periods is degreased and weighed. Drugs and cosmetics are considered to have good efficacy when the weight of the second sample is more than the weight of the first sample [10].

## Unit area trichogram

The unit area trichogram is a semi-invasive method for scalp hair, which estimates three main growth parameters: hair follicle density, proportion of anagen/telogen fibres, and hair shaft diameter. It is based on plucking hair from a defined area (usually 60 $mm^2$). The hairs are assessed clinically and microscopically. The unit area trichogram may be used for follow-up of scalp hair changes in clinical studies, for observing hair growth cycling, and for monitoring topical or systemic drug and cosmetic effects. This test has the disadvantage of being dependent on correct sampling [11, 12]. For this reason, the classic trichogram (as describe below) is more useful for clinical practice.

## Trichogram

The trichogram is a semi-invasive microscopic method, which is most commonly used to evaluate hair loss in clinical practice in Europe. It allows the analysis of the proportion of hair in different phases of the hair cycle [12].

The patient should not wash their hair for three days prior to the examination. About 100 hairs are plucked from the scalp using rubber-armed forceps. The hairs are removed with one, quick, forceful pull perpendicular to the scalp and always along the direction of hair growth. In most cases two sites are investigated. The

first site is 2 cm off the frontal line and 2 cm off the midline. The second site is in the occipital region, 2 cm lateral from the protuberans occipitalis. In alopecia areata, the first site would be in close proximity to an alopecia patch and the second in the contralateral, clinically unaffected side. There is a variant of performing a trichogram, in which hairs are plucked from four sites (frontal, occipital, right and left parietal). The hair roots are evaluated under light microscopy to determine the number of hairs in the different phases of the hair cycle. The results are given as a percentage of the total number of hairs being evaluated. Normal values are: anagen hairs 66–96%, catagen hairs 0–6%, telogen hairs 2–18%, and dysplastic/ dystrophic hairs 0–18% [13]

The trichogram is most useful in the diagnosis of acute telogen effluvium. In such cases the percentage of telogen hairs is significantly increased and can be double the upper limit [2, 13]. The increased percentage of dystrophic hair and slightly increased percentage of telogen hairs accompanied by features of hair miniaturization is noticed in androgenic alopecia, but the results of the trichogram are not clear-cut for the diagnosis of this disease [14]. An additional barrier in diagnosing androgenic alopecia with this method is the fact that technically vellus hairs, a hallmark of androgenic alopecia, are not plucked for investigation because they are too short to be plucked together with the bundle of terminal hairs.

The trichogram may also be applied for treatment monitoring, in particular in patients with telogen effluvium [13, 15]

## Phototrichogram

The phototrichogram is a non-invasive method which is based on sequential macro photographs of a selected scalp area. This method is based on the notion that anagen hairs grow at a rate of about 1 mm every 3 days, while in the same time catagen hairs will show only moderate elongation and telogen hairs will not grow at all [16, 17] For the evaluation, 1–3 areas of about 1 $cm^2$ each are shaved. This area is then photographed. After 3 days a second photograph is taken and the proportion of growing (anagen) hairs is evaluated.

As in the classic trichogram, a significantly increased proportion of telogen hairs is indicative of acute telogen effluvium [13, 18].

This method is not widely used because of its limited value in broad hair diagnostics, possible inaccuracy in sampling, and because patients with hair loss are reluctant to have their hair shaved.

## Trichoscan

Trichoscan is based on the same theoretical foundation as the phototrichogram. [19, 20]. There are, however, two major differences. The technique is automated, based on software which was developed for image evaluation, and dermoscopy photographs are used instead of conventional photography. To enhance hair visibility, the inspected area is dyed with a colouring solution (e.g. Goldwell top chic, black 2N, Darmstadt, Germany) [13].

This method has similar indications as the classic trichogram. It has the advantage of being automated, but the disadvantage of not being sensitive to hair shaft abnormalities, thin regrowing hairs in telogen effluvium, or merely debris from the hair dye [14].

## Trichoscopy

Trichoscopy is a method of hair image analysis based on dermoscopy or videodermoscopy of the hair and scalp [21, 22]. It allows the visualization of hair and hair follicle ostia at high magnification and the measurement of relevant trichologic structures. The usual working magnifications are 20- and 70-times; however, a handheld dermoscope, which gives 10-times magnification, may be sufficient in many clinical situations.

A regular trichoscopy screening includes evaluation of the hair and scalp in the frontal, occipital, and both parietal areas. In selected cases other locations are chosen for trichoscopy. These include eyebrows, eyelashes, or body hairs in other areas.

Trichoscopy allows the distinction between normal terminal hairs and vellus (or vellus-like) hairs, which by definition are 0.03 mm or less in thickness. The method enables visualization of micro-exclamation hairs which may be 1–2 mm or less in length and structural hair shaft abnormalities. The number of hairs in one pilosebaceous unit may be assessed [23]

It may be seen whether hair follicles are normal, empty, fibrotic ("white dots"), filled with hyperkeratotic plugs ("yellow dots"), or contain destroyed hair remains ("black dots") [24]. Cutaneous miscovasculature and its abnormalities may be appreciated in scalp skin.

In androgenic alopecia, trichoscopy allows visualization of abnormalities, which are known from research, performed with invasive and semi-invasive techniques. According to criteria developed by Rakowska et al., female androgenic alopecia may be differentiated from chronic telogen effluvium based on the following trichoscopy criteria [25]. The major criteria are the

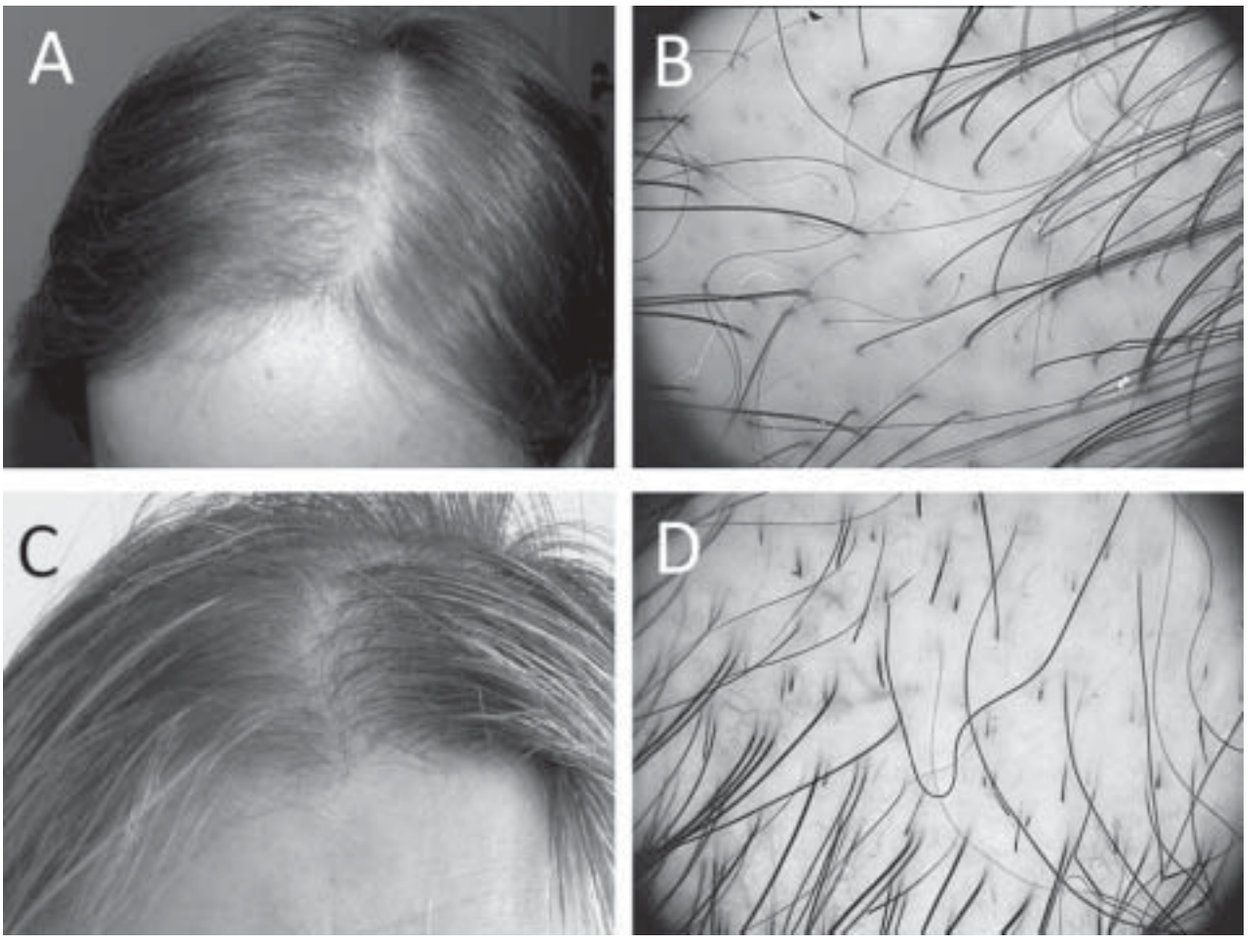


**Figure 1.** *Trichoscopy is a new technique, which allows non-invasive differentiation of hair disorders.In androgenic alopecia (**A**,**B**) most prominent is hair shaft thickness heterogeneity and presence of vellus hairs. In diffuse alopecia areata (**C**,**D**) micro-exlamation mark hairs and tapered hairs domine in the in the picture and black dots are present. In both diseases multiple yellow dots are visible, what differentiates these diseases from other causes of hair loss*

**Rycina 1.** *Trichoskopia jest nową techniką pozwalającą na nieinwazyjne różnicowanie łysienia. W łysieniu androgenowym (**A**, **B**) dominuje zmienna grubość włosa i obecność włosów ścieńczałych. W rozlanym łysieniu plackowatym (**C**, **D**) na zdjęciu dominują włosy wykrzyknikowe i zwężające się, obecne są czarne punkty. W obu chorobach widoczne są liczne żółte punkty, co odróżnia je od innych przyczyn łysienia*



ratio of: 1. more than 4 yellow dots in 4 images (70-fold magnification) in the frontal area, 2. below average hair thickness in the frontal area compared to the occiput, and 3. more than 10% of thin hairs (below 0.03 mm) in the frontal area. Minor criteria encompass increased frontal to occipital ratio of: 1. single-hair pilosebaceous units, 2. vellus hairs, and 3. perifollicular discoloration. Fulfilment of 2 major criteria or 1 major and 2 minor criteria allows the diagnosis of FAGA, based on trichoscopy, with a 98% specificity.

For trichoscopy diagnosis of alopecia areata, evenly distributed yellow dots (hyperkeratotic plugs) and short vellus hairs are the most sensitive markers and black dots, tapering hairs (or micro-exclamation mark hairs), and broken hairs are the most specific markers [26, 27] (Fig. 1).

There are no specific trichoscopy features of telogen effluvium; however, this disease may be suspected based on short, dark regrowing hairs and the presence of hair follicles in the absence of features of alopecia areata.

Ectodermal dysplasia, which may be associated with a variety of endocrine abnormalities, may be suspected based on abnormalities in hair shaft structure and colour, as visualized in trichoscopy.

## Light microscopy

Light microscopy may be useful to directly assess the hair shaft, its anomalies, and thickness. This method is rarely used but is particularly useful for the evaluation of genetic hair dystrophies [28].

## Polarized light microscopy

Polarized light is light in which all the rays oscillate in one plane. A polarizing microscope has two disk accessories. They are made up of polarizing plastic that allows light oscillating in one plane to pass. One of them is called the polarizer (placed below the condenser). Another similar disc is placed in the top part of the microscope and cuts off all the light oscillating in a perpendicular plane. This disc is called the analyzer. The placement of the discs is such that they allow light to pass vibrating in planes perpendicular to each other, which allows the visualization of some hair shaft abnormalities.

Polarized light microscopy is of particular value in trichothiodystrophy, where the characteristic alternate dark and white bands of hair shaft can be seen. This sign is known as a "tiger tail" appearance, which is not visualized under light microscopic examination [29].

## Pathology

Pathology examination of a scalp biopsy is an invasive technique, but remains a standard in diagnosing hair loss. The biopsy is usually performed with a 4-mm cylindrical punch. Some pathologists suggest performing at least 3 biopsies [30]: two from the affected site (one for vertical and one for horizontal evaluation) and one from a non-affected site (for horizontal evaluation only). Clinicians usually prefer to perform one biopsy, and another only if needed for diagnostic purposes. Increasingly, trichoscopy is used to choose the proper area for scalp biopsy. Pathology enables evaluation of hair follicle structure, anagen and telogen hairs, hair follicle miniaturization, perifollicular infiltrates, and other abnormalities in the affected skin.

## Immunopathology of hair follicles

Immunopathology of hair follicles may be performed to visualize selected molecules within the hair follicle [31, 32]. This method does not appear to be useful for diagnosing hair loss in endocrine disorders.

## Confocal microscopy

Reflectance confocal laser scanning microscopy, also known as reflectance confocal microscopy (RCM), is an optical technique that allows non-invasive imaging of the upper portion of the skin at a resolution that permits visualization of cellular, with near histological resolution, details in real time. With a penetration depth of about 200 micrometres, the technique allows non-invasive imaging of the epidermis and upper dermis and distal parts of hair follicles and hairs. The system generates 500 × 500 micrometre fields of vision from a total 8 × 8mm area, which may be analyzed in a single session. These optical sections may be evaluated in horizontal blocks, vertical stacks, or "cubes", giving a pseudo-3D visualization of analyzed structures [33, 34].

RCM has been used for the assessment of benign and malignant pigmented lesions, in particular for early diagnosis of melanoma. Recent studies showed the potential application of this method in evaluating patients with hair loss [35]. It has been shown in case studies that this method may be of benefit as a supporting tool in diagnosing alopecia areata, androgenic alopecia, and genetic hair dystrophies (Fig. 2). The advantage of this method is the highest possible magnification in non-invasive skin imaging. A disadvantage is the small width of the visualized area, and thus the risk of inadequate sampling. New

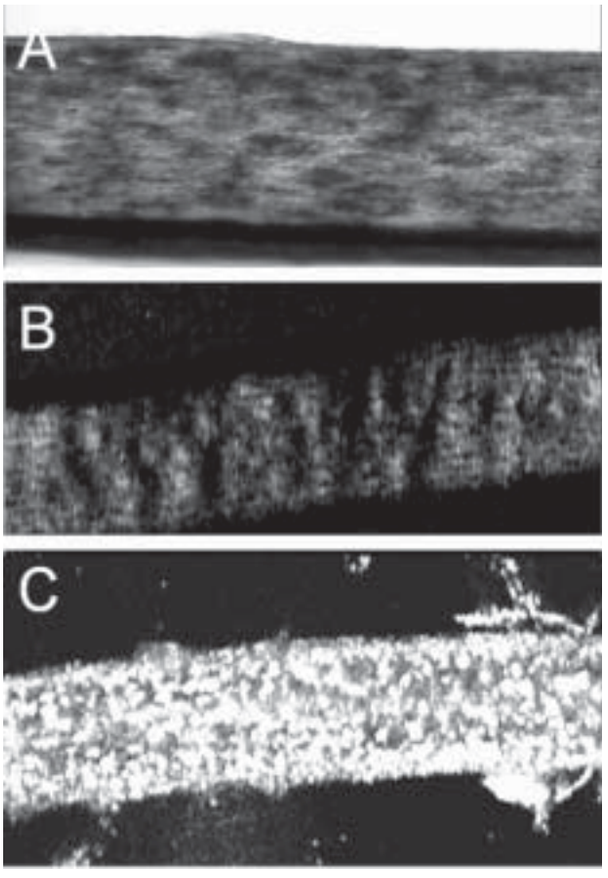


**Figure 2.** *Hair shaft of a patient with trichothiodystrophy, which may be associated with hypogonadism and other developmental abnormalities, shows no significant abnormalities in light microscopy (**A**), a characteristic „tiger-tail" stripes in polarized ligh microscopy (**B**) and a grainy structure in reflectance confocal microscopy (**C**)*

**Rycina 2.** *Obrazy fragmentu włosa pacjenta z trichotiodystrofią, która może towarzyszyć hypogonadyzmowi i innym zaburzeniom rozwojowym nie wykazuje żadnych zmian w mikroskopie świetlnym (**A**), charakterystyczne paski „ogonu tygrysa" w świetle spolaryzowanym (**B**) i ziarnista struktura w refleksyjnym mikroskopie konfokalnym (**C**)*

RCMs are equipped with a videodermoscope to allow better selection of the area to be evaluated.

## Conclusions

In conclusion, diagnosing hair loss is a complex and sometimes time-consuming process. However, detailed hair examination may occasionally lead to the suspicion of an endocrine disorder. Diagnosing hair loss in patients with an established diagnosis of an endocrine disorder is essential to exclude other coexisting causes of hair loss in clinical practice. Current hair evaluation techniques allow precise assessment of hair loss and may also serve as a sensitive parameter in clinical trials.

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