Biochemical markers, sometimes referred to as biomarkers, are molecules found in certain parts of the body, including bodily fluids such as blood, urine and saliva. They can be measured to determine normal or abnormal biological function and help to identify the presence of a disease or condition. In pituitary tumors, such as Cushing's disease and acromegaly, biochemical markers play an important role in diagnosing patients and monitoring the status of their disease.

Currently, there are no uniform assays, or tests, used to measure the biochemical markers of Cushing’s disease and acromegaly. One of the long-term goals of the Pituitary Alliance is to establish partnerships to standardize biochemical marker assays for the diagnosis and monitoring of these pituitary tumors to help patients better manage their disease and improve quality of life.

**Cushing’s Disease**

Cushing’s disease is caused by excess production of the biochemical marker cortisol, which is triggered by an adrenocorticotropic hormone (ACTH)-secreting pituitary tumor. Excess cortisol can result in many physical, hormonal, metabolic and cardiovascular complications. The effects of excess cortisol circulating in the body contribute to severe illness and death, with mortality up to four times higher than in the healthy population.

There are typically multiple steps taken to confirm a diagnosis of Cushing’s disease. The first step is to diagnose the excess levels of cortisol, known as hypercortisolism or Cushing’s syndrome. The second step is to confirm the source of excess cortisol. This may include determining the presence of an ACTH-secreting adenoma through measurement of ACTH or conducting imaging tests, including a magnetic resonance imaging (MRI) or a computed tomography (CT) scan. **Urinary-free cortisol** and **late-night salivary cortisol** are two tests that can be used to diagnose hypercortisolism and subsequently to monitor biochemical control in patients with Cushing’s disease.

- **Urinary-Free Cortisol (UFC):** UFC is a measure of cortisol in the urine that has been used by the medical community for more than 40 years.
  - The patient must collect every urine sample throughout a 24-hour period, keep them refrigerated and provide them to a laboratory for testing. Based on these complexities associated with administration, oral and written instructions for the test may need to be provided to the patient.
  - Cortisol levels in the urine can vary, therefore at least two UFC tests are recommended to confirm a hypercortisolism diagnosis. UFC tests are also used for monitoring biochemical control of the disease.
  - Since UFC reflects renal filtration, values can be significantly lower in patients with moderate to severe renal impairment and result in a false negative.
  - A key benefit of the UFC test is that it only measures the type of cortisol that causes hypercortisolism, called circulating, free cortisol. It does not measure bound cortisol that can be increased by certain conditions and medicines.

- **Late-Night Salivary Cortisol:** Late-night salivary cortisol is a test that is conducted by taking a saliva sample before bedtime, typically between 11:00 PM-12:00 AM depending on a person’s schedule.
  - Normally, cortisol levels are lowest at nighttime, but in patients with hypercortisolism, levels may remain high.
  - Similar to UFC, it is recommended that a late-night salivary cortisol test be conducted on two separate occasions to confirm a diagnosis. Late-night salivary cortisol tests are also used for monitoring biochemical control of the disease.
  - A late-night salivary cortisol test may be more convenient than UFC since only one collection per test is needed.
The late-night salivary cortisol test may be more sensitive to detecting mild forms of hypercortisolism than UFC.

Late-night salivary cortisol tests may be less accurate in patients who smoke, use chewing tobacco or recently ate licorice due to the presence of an enzyme found in these substances that converts into cortisol. Additionally, the test may not be appropriate for those with variable bedtimes due to abnormal circadian rhythm, which can impact nighttime cortisol levels.

Various methods have been used to measure cortisol in the saliva, resulting in different reference ranges and yielding differences in sensitivity and specificity. Standardization for this test is needed.

Acromegaly

Acromegaly is a rare chronic endocrine disorder that occurs when excess amounts of the biochemical marker growth hormone (GH) are produced. The majority of acromegaly cases are caused by a non-cancerous tumor on the pituitary gland that secretes excess GH, leading to elevated levels of insulin-like growth factor-1 (IGF-1). The overproduction of IGF-1 results in musculoskeletal changes and hormonal, heart and metabolic complications. Excess IGF-1 in patients with acromegaly contributes to high rates of heart disease, which is responsible for approximately 60% of deaths in this population. Tests used to diagnose acromegaly include, but are not limited to oral glucose tolerance test, random GH test and serum IGF-1. Serum IGF-1 tests are used to measure GH and IGF-1 in any acromegaly patient, while oral glucose tolerance tests are used to measure biochemical markers in patients only after surgery.

- **Oral Glucose Tolerance Test (OGTT):** For almost 40 years the OGTT has been a standard measurement of GH in acromegaly.
  - Following ingestion of a glucose drink, GH levels in normal patients should be suppressed, but in patients with acromegaly, oral glucose does not suppress GH.
  - Diabetes, liver and kidney disease, anorexia and puberty may impact results of this test.
  - The need for standardization of this test remains since there are no data comparing the dose of glucose, variables such as age, sex and weight which can affect GH are not taken into account and commercial assays have varying results.

- **Random GH Test:** GH tests are used to help identify excess or diminished GH production and give the doctor information about the severity of a person's condition.
  - GH can also be measured to assess the success of therapy for acromegaly.
  - GH levels vary naturally depending on factors such as age, the time of day and time of last meal. To account for the variation in GH levels, your doctor may average the values from a series of “random” blood tests.
  - Liver disease, malnutrition and uncontrolled diabetes can elevate GH levels and subsequently affect the results of this test.
  - The lack of a well-defined normal or safe range has minimized the effectiveness of random GH testing.

- **Serum IGF-1:** Serum IGF-1 is a test that measures the levels of IGF-1 in the blood.
  - The serum IGF-1 test can be conducted any time of the day without fasting.
  - Oral estrogen medicines, pregnancy, systemic illness, including catabolic states (i.e., when the body breaks down proteins resulting in muscle degradation), malnutrition and diabetes can impact results of this test.
  - Serum IGF-1 is typically the ideal test for diagnosis since the absence of elevated IGF-1 levels in acromegaly is rare. However, standardization of serum IGF-1 is needed as there is a lack of agreement within the medical community on the use of different assays and validated normal ranges of this biochemical marker.