Phenylketonuria (PKU; OMIM 261600) is an inborn error of metabolism caused by mutations in the phenylalanine hydroxylase (PAH) gene. The mutations lead to a decrease in enzymatic activity of PAH, which is predominantly a hepatic enzyme. PAH requires the cofactor tetrahydrobiopterin (BH4) to convert phenylalanine (Phe) to tyrosine (Tyr). Therefore, if there is a deficiency either in PAH or its cofactor BH4, then the result is the accumulation of excess phe in the blood and brain.

Recently neonatal screening programs for PKU enable physicians to identify most of the cases, and for early interventions to prevent
severe consequences in later years of life. In cases of inadequately controlled blood Phe, neurocognitive findings, such as low intelligence quotient (IQ), decreased attention span, poor executive functions (such as planning, working memory, inhibition, flexibility, and behavioral issues), and psychosocial effects are observed in later childhood, and they extend into the adulthood.²

The prevalence of PKU varies worldwide. In Europe, the mean prevalence is approximately 1:10,000 newborns, and it is most commonly encountered in Ireland and Turkey whereas less commonly in Finland.³ It is reported that the disease prevalence in Turkey is one in every 4000 births that is believed to be caused partly by the high rate of consanguinity within the population.⁴ According to the statistics of the Turkish Ministry of Health, PKU prevalence is one in 6228 births, which is still one of the highest prevalence rates in the world.⁵

A group of pediatric metabolism specialists who are well-known for their clinical experience in the management of PKU patients were invited to discuss major issues and offer a standard for diagnosis, treatment, and follow up for PKU as well as to determine and inquire into specific national problems in hindering treatment success. Since the management of PKU shows wide variations across Europe⁶, and in the world⁷, the committee members decided to prepare a recommendation paper for clinicians of interest for PKU management for Turkish patients of all age groups. For this purpose, face-to-face advisory board meetings were held twice in December 2016 and December 2017 on twelve selected topics in PKU management. They discussed unmet needs in diagnosis, treatment, and follow-up of PKU patients in Turkey. Recommendations about discussion points were collected and advisory board reports were prepared for each meeting. After the participants reviewed the reports, an online update meeting was held for the final decisions about the statements, and conclusions in January 2021. The authors of this paper have suggested the key clinical recommendations that should be prioritized for implementation for PKU in Turkey.

Discussion Topics

Diagnostic cut-off levels for PKU

Similar to published recommendations and guidelines in PKU management, neonatal dried blood spot (DBS) screening, performed within the first 48-72 hours of life, is the crucial step in the diagnosis, and early initiation of treatment. Although it is recommended in the literature that blood samples for PKU screening should be obtained after 72 hours of birth, the samples are sometimes obtained within 24 hours in Turkey, because the newborns are discharged early due to an overload of maternity hospitals. If the blood is sampled before the second day of life or without sufficient feeding of the baby, a second sample should be taken on the 5th-7th days of life. Hyperphenylalaninemia (HPA) is defined as any blood Phe ≥2 mg/dl (≥120 μmol/L).³ Therefore, it is obligatory to have DBS from each newborn, and the procedures are defined by the legislation of the Ministry of Health of the Turkish Republic. Filter paper for newborn screening for PKU is present at all healthcare units and hospitals. After samples are obtained on the filter paper, they are sent to two centers affiliated with the Ministry of Health of the Turkish Republic. If the results of Phe determination are suspicious, then newborns are immediately referred to expert metabolic centers for the determination of blood Phe levels, and confirmation of the diagnosis of HPA. The current algorithm is given in Figure 1.⁵

The committee members discussed that the cut-off level for blood Phe was 3 mg/dl (180 μmol/L) previously in Turkey and had then been decreased to 2 mg/dl (120 μmol/L). It is also important to emphasize that even blood value below 2 mg/dl alone may be misleading in some cases (i.e. 1.98 mg/dl) because complete and correct management of PKU depends on laboratory findings integrated with clinical symptoms and signs.
Statement #1: Cut-off level for DBS should remain at 2 mg/dl (120 μmol/L).

Classification criteria for PKU

There is no consensus in the literature about patients with blood Phe concentrations >10 mg/dl (>600 μmol/L) should be treated who do not receive any treatment. However, Phe level between 2 and 6 mg/dl is generally accepted as hyperphenylalaninemia (HPA), 6-10 mg/dl as mild; 10-20 mg/dl as moderate; and >20 mg/dl as severe PKU.

Since blood Phe levels may increase with age, patients with Phe levels <360 μmol/L should be monitored (at a lower frequency) during the first year of life at minimum. The evidence regarding initiation of treatment with blood Phe levels between 6 and 10 mg/dl (360 and 600 μmol/L) is incoherent. Waisbren et al. reported a decreasing trend toward of intelligence quotient (IQ) in those with higher Phe levels after having compared 3 groups (<400, 400–500 and >500 μmol/L), and recommended that treatment should maintain Phe levels <400 μmol/L throughout childhood in all PKU forms. It was predicted that for every 1.66 mg/dl (100 μmol/L) increase in mean Phe levels, IQ would decrease by approximately 6 points.

Based on evidence from small size studies, it is recommended in the European (EU) guidelines that patients without treatment and who have the Phe concentrations between 6 and 10 mg/dl (360 and 600 μmol/L) should be treated during the first 12 years of age particularly to provide a good metabolic control during childhood to prevent cognitive function impairment.

In a recent single-center study in Turkey, cognitive functions and attention-related problems were compared between healthy children and untreated patients with HPA. A total of 60 children were recruited to the study, 41 hyperphenylalaninemia patients aged 6-16 years with untreated blood Phe levels between 240 and 600 μmol/L and 29 healthy controls. The children with untreated Phe levels between 240-360 μmol/L compared to their healthy peers, and the children with Phe levels 360-600 μmol/L compared to the children with Phe levels between 240-360 μmol/L were found at higher risk for cognitive and attention-related...
problems. The outcome indicates that the “safe” upper Phe level should be considered to be lowered.\(^8\) Considering Turkish PKU patients, the experts have decided that <6 mg/dl level indicates mild disease and patients can be followed up closely without treatment if their clinical pictures denote no sign of the disease. However, it is agreed that a decimal system should be accepted for the classification of patients in the national PKU database such as 6-10 mg/dl mild, 10-20 mg/dl moderate, and >20 mg/dl severe. It is determined that this method will be easy to use and help better classify PKU patients.

The Phe tolerance is the amount of Phe per kg of body weight or mg/day to maintain the blood Phe concentrations within the target range. This may also be described as natural protein tolerance expressed as grams per day.\(^3\) In PKU, the individual dietary Phe tolerance is influenced by many factors such as severity of the clinical picture, net protein catabolism-synthesis ratio, energy intake, dosage and distribution of Phe-free L-amino acid supplements, and target blood Phe concentrations.\(^3\) Children with moderate or severe PKU usually tolerate 200–500 mg/day of phenylalanine. Phe requirements are highest in early infancy ranging from 55 mg/kg/day at 0–3 months of age to 27 mg/kg/day at 12 months.\(^3\) After the age of 1 year, there is a slow and steady decline in tolerance per kg of body weight. It has been recognized that children with classic PKU usually only tolerate between 200 and 500 mg Phe/day. Patients with a milder form of PKU, namely those with untreated blood Phe concentrations less than 16.6-20.0 mg/dl or 1000–1200 μmol/L, usually tolerate ≥500 mg/day of dietary Phe. By comparison, the third US National Health and Nutrition Examination Survey (NHANES III) showed that mean daily dietary Phe intakes for all ages and gender groups in the population without PKU was as high as 3400 mg/day.\(^3\) A clear relationship between Phe tolerance at 2 years and 10 years of age was found.\(^9\) However, it is unclear how this tolerance relates in older patients aiming to achieve a target blood Phe of 120 to 600 μmol/L. All patients’ Phe tolerance should be evaluated periodically, especially during at times of rapid growth, changes in body composition, or use of different treatment modalities [e.g. tetrahydrobiopterin (BH4)].\(^3\)

Experts have discussed which method will provide a more robust classification for PKU patients, blood Phe levels at the baseline or Phe tolerance. Considering the literature data, and achieving rapid and sustained treatment success, it is recommended that the national database should be classified both according to Phe levels and tolerance.

**Statement #2:** PKU patients can be classified according to blood Phe levels as 6-10 mg/dl mild, 10-20 mg/dl moderate, and >20 mg/dl severe cases. Classification due to Phe tolerance should be taken into account for individualized treatment for each PKU patient.

**Phe levels for treatment initiation**

In UK recommendations\(^6\), USA guidelines\(^7\) and EU guidelines\(^3\), it is reported that PKU treatment should be started as early as possible, preferably within the first week of life to have blood Phe in the treatment range within the first 2 weeks of life. Generally accepted blood Phe level for initiation of diet is 6 mg/dl (360 μmo/L).\(^8\) Still there is no current consensus on whether to initiate treatment between 6-10 mg/dl (360-600 μmol/L) because evidence regarding the clinical outcome in untreated patients within these limits is not definite.\(^10-12\) However, the cut-off value to initiate treatment is accepted as 6 mg/dl (360 μmol/L) both in USA and EU guidelines.\(^3,7\)

We decided to determine a definite cut-off level for treatment in Turkey because there are cases with no treatment up to 10 mg/dl in some centers. It is considered that the most important argument point is the definition of levels according to the patients’ age because it is easier to manage blood levels when the diet is completely managed by parents. However, patients are expected to take responsibility for
their diseases during adolescence which may be quite a difficult period of their lives.

Blau et al.\textsuperscript{13} reported that deciding the target blood Phe concentrations during the follow-up had no standard in patients with PKU. According to treatment policy data from 17 countries, the lower target Phe levels for PKU patients were in the range of 0.7-2.\textsuperscript{14} mg/dl (40 to 130 μmol/L). During the lifetime period, the upper target blood Phe concentrations were recommended in two to five stages by different countries. It is defined in three stages in Turkey; <240 μmol/L for 0-2 years of age, <900 μmol/L for 3-15 years of age and <1,200 μmol/L for ≥16 years of age.\textsuperscript{14}

**Statement #3:** National cut-off value for treatment can be accepted as 6 mg/dl (360 μmol/L).\textsuperscript{*}

* Considering increased risk in executive and neurocognitive functions, experts at one center prefer to initiate treatment at 4 mg/dl (240 μmol/L). Follow-up is the preferred approach only in selected cases with mild PKU (personal communication).\textsuperscript{8}

**Age limit for treatment initiation**

Similar to current guidelines\textsuperscript{3,6} and recommendations\textsuperscript{5} the committee members have agreed on the initiation of treatment as soon as possible ideally before 10 days of life for prompt control of neurocognitive functions. Examples have been given concerning late-diagnosed PKU patients, who have improved outcomes after initiation of nutritional therapy. It is also emphasized that the number of late-diagnosed PKU cases is increasing now in Turkey due to Syrian refugees on the Southeast border, although there is not enough published data (personal communication).

It is concluded that high Phe levels should always be treated no matter what the clinical picture indicates.

**Statement #4:** Treatment should be initiated as soon as PKU is diagnosed without considering age.

**Phe limits according to age groups**

The evidence for patients <12 years of age is strongly indicating that a Phe concentration of 6 mg/dl (360 μmol/L) should be considered as the upper target Phe concentration. There are also some articles indicating that the upper target Phe levels need to be lower in this age group, but at present, the evidence to lower the upper target for Phe is not robust enough. If possible, a meta-analysis of the available data should be performed to investigate the relationship between neurocognitive and neuropsychological outcome and blood Phe concentrations. Also, it should be examined whether higher Phe levels other than 360 μmol/L would result in better results. This requires an international collaborative work.\textsuperscript{3}

According to EU guidelines, the evidence for patients >12 years of age is mainly indirect, because there are no studies investigated the effect of Phe levels during adolescence in patients with good metabolic control during childhood. Taking into account the lower grade of evidence, an upper target Phe level at 600 μmol/L between ages 12 and 18 years is recommended.\textsuperscript{3}

During adulthood, it is reported that the main troubling issue is the determination of PKU outcomes associated with the increased Phe levels. There are no large-size controlled longitudinal studies to determine the optimal upper target blood Phe levels for adults. Therefore, further data collection is required for definite recommendations.\textsuperscript{3}

The committee members have explained that Phe levels are determined generally be high after the age of 1-2 years until adulthood. The underlying causes are described as low treatment compliance due to low socioeconomic
levels, and cultural factors. It is decided that it will be more practical for clinical practice if target Phe levels are defined according to patient age groups.

**Statement #5:** Three-stage upper target Phe concentration is most convenient for our country. PKU patients should be divided into three main age groups: newborn to adolescence (<240 μmol/L), adolescence to adulthood (10-16 years/<360 μmol/L), and adulthood (<600 μmol/L). Although it may be difficult to achieve these thresholds due to the rapid growth and active daily life of patients especially during the school-age, treating physicians should try to achieve normal Phe levels as soon as possible.

**Monitoring frequency of Phe levels according to ages**

Close monitoring (at least weekly) is recommended in newborns up to age 1 with increased surveillance during periods of rapid growth and transitions of diet, such as with the introduction of solid foods. However, recommended monitoring frequency differs after the first age: biweekly to monthly up to 12 years of age in USA guidelines, twice weekly in EU guidelines, and biweekly until school entry in UK recommendations.

Experts have discussed problems of blood sample collection, outpatient clinics, and laboratory load in different centers. Since Turkey has one of the highest disease prevalences, inadequate infrastructure capacity may present obstacles to efficient healthcare service both for PKU and other patients. It is further discussed that equipment capacity, trained healthcare personnel, and materials for testing are commonly insufficient in such cases.

Another noteworthy issue is the demographic characteristics of parents of children with PKU. They are mostly undereducated and have low socioeconomic status, so it may be quite difficult to help them understand the disease and the necessity of a specific and lifelong diet therapy even when interviewed face-to-face. Therefore, although home monitoring is a practical method and is being performed in some countries with a low number of PKU patients and a high socioeconomic state, it may be misleading for some Turkish patients. Physicians may not be sure if the Phe level is increased due to the wrong sample collection method or requirement for new diet adjustments. Moreover, there are important drawbacks to PKU patient follow-up in Turkey. The main ones are the absence of a registry system for dieticians, an increased number of patients, and the absence of a call center follow-up system. The committee members have decided that PKU patients should be monitored in well-equipped metabolic centers by trained medical personnel. However, this is a major insufficiency in the monitoring of Turkish PKU patients. Under these circumstances, it is admitted that even weekly follow-up of infants cannot be carried out in some reference centers.

In EU guideline, the minimum frequency of blood Phe measurements and outpatient clinic visits for each age group are listed (Table I and II).

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1 year</td>
<td>Weekly</td>
</tr>
<tr>
<td>1-12 years</td>
<td>Fortnightly</td>
</tr>
<tr>
<td>&gt;12 years</td>
<td>Monthly</td>
</tr>
<tr>
<td>Pregnancy preconception</td>
<td>Weekly</td>
</tr>
<tr>
<td>During pregnancy</td>
<td>Twice weekly</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1 year</td>
<td>Every 2 months</td>
</tr>
<tr>
<td>1-18 years</td>
<td>Twice per year</td>
</tr>
<tr>
<td>&gt;18 years</td>
<td>Once per year</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Once per trimester</td>
</tr>
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</table>
Since each age group has its specificities, such as starting school, changing school, puberty problems, and each country has its facilities and resources for healthcare, it is resolved that individualization of monitoring frequency may be accepted. The follow-up frequency of blood Phe measurements for Turkish patients is presented in Table III. These are the recommended frequencies for an office visit with a pediatric metabolic diseases specialist because the growth and vaccination program of all children are managed by family physicians and general pediatricians in Turkey. Also as the daily workload of pediatric metabolic diseases specialists is quite high in our country, the effective activation of the reference system is the best way for the prioritization of PKU patients.

**Statement #6:** Individualized frequency for blood Phe levels may be endorsed for some PKU patients. However, compliance with an ideal follow-up frequency list is strongly recommended.

**PKU treatment requires interdisciplinary teamwork**

In 2017, it was recommended in the “The complete European guidelines on phenylketonuria: diagnosis and treatment” paper that all patients with PKU should be treated at specialized metabolic centers having specialized laboratory facilities. It is stated that the minimum number of health professionals within an interdisciplinary team for patients of all ages should be a metabolic physician and a dietician with experience in inherited metabolic disorders (IMD), and it is strongly advised to have a (neuro)psychologist and social worker on the team. Along with the core team, support from other medical professionals such as pediatricians, biochemists, nurses specialized in IMD, and genetic specialists and support groups may be included in the management of PKU patients.

The process of transferring children to adult care is another important issue in patient management. The transfer should be a carefully structured ‘transitional’ process, beginning from around the age of 12 years. The patient should take over the lead of disease management from parents/caregivers. The patient-controlled process must occur even if the patient is staying at the same pediatric service. It is recommended that patients and families should have an individualized care plan and timetable for transition, together with detailed information about the adult center. This should be jointly agreed upon and written down with teenagers, caregivers, and health professionals. This plan should include treatment goals, a timetable for transfer, and ensure there is a consistent approach between all health professionals. It should also provide a mutual understanding of the transition process. It has been demonstrated in PKU, that most patients can make a successful transition to adult care with careful planning, close liaison between pediatric and adult teams, and patient and caregiver involvement. There is no right time or age for the subsequent transfer of patient care to the adult treatment center to occur but is commonly between 16 to 18 years of age, although some flexibility may be required depending on the maturity and circumstances of the patient. Unfortunately, there is no established transition system in Turkey, so patients who are diagnosed at pediatric age continue being followed by their first physicians. The committee members would like to emphasize that a system is crucial for

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
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</thead>
<tbody>
<tr>
<td>&lt;1 year</td>
<td>Once a month (once a week in breastfeeding infants)</td>
</tr>
<tr>
<td>1-6 years</td>
<td>Every 2 months</td>
</tr>
<tr>
<td>7-10 years</td>
<td>Every 3 months</td>
</tr>
<tr>
<td>11-16 years</td>
<td>Twice in a year</td>
</tr>
<tr>
<td>&gt;16 years</td>
<td>Once a year</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Once a week</td>
</tr>
</tbody>
</table>
uninterrupted treatment during the transition period.

Method for Phe determination

In the 1960s, Guthrie developed a simple semi-quantitative test to detect hyperphenylalaninemia (HPA) in large populations. However, newborn screening (NBS) for PAH deficiency in the United States is now primarily performed by tandem mass spectrometry (MS/MS). It is accepted in the USA that MS/MS-based NBS is far more accurate in determining blood Phe concentration than older screening methods. According to EU guidelines, fluorometric enzyme analysis is more reliable than the Guthrie test, but more recently amino acid concentrations are usually measured by high-performance liquid chromatography (HPLC) and tandem mass spectrometry which are more precise. Studies indicate that differences between these two methods are relatively small. Committee members discussed that there is more deviation in tandem mass spectrometry, and a consensus should be reached about the method. One of the critically important issues is that cut-off levels of NBS laboratories are not standardized nationwide, because laboratories are not under the control of a national quality control system. It is underlined that different laboratory results may be misleading for daily clinical practice.

Statement #7: HPLC is the most reliable method for the determination of blood Phe levels.

Crucial nutritional issues: how much natural and how many protein substitutes should be used?

A low Phe diet should be initiated at the time of diagnosis for PKU patients. The PKU diet mainly consists of fruits and vegetables low in Phe, variable amounts of low-Phe special foods, and Phe-free protein substitutes providing essential amino acids (especially tyrosine), vitamins, and minerals. There are many difficulties in the nutritional management of PKU because firstly it should be lifelong. Secondly, it is complex and time-consuming. Thirdly, it requires knowledge of foods and recipes, as well as cooking skills, and continuous food measurement. The constraint of a diet that is ultimately focused at the threshold of a calculated Phe intake, and an astringent diet bears the risk of micronutrient deficiencies, such as iron, zinc, selenium, and vitamin B12.

For infants with PKU, human breast milk should be given greater consideration in PKU than is currently granted, because it has greater biological and developmental advantages. Therefore, it is recommended by the World Health Organization (WHO) that breastfeeding combined with the prescribed amount of an infant Phe-free protein substitute should be promoted. Protein substitutes are available as amino acid powders, gels, ready-to-drink liquids, and tablets/capsules. Ready-to-drink liquids are suitable for children from 3 years of age. In the case of adult PKU patients, the long-term use of ready-to-drink liquid protein substitutes is associated with better compliance by lowering blood Phe and improving nutritional biochemical markers.

Preparation of low Phe diet from natural foods and protein substitutes should be diligently performed. In most patients, precursor-free L-amino acids will likely supply 52 to 80% of the total protein intake. But, the optimal amount of L-amino acids is still undetermined. The recent Cochrane review concludes there was insufficient data to define a conclusion regarding the dosage of Phe-free L-amino acid supplements for PKU treatment.

It is reported in the EU guidelines that many centers in Europe and beyond prescribe L-amino acids/total protein between 2 and 3 g/kg/day in infants aged 0–1 y; 1.5–2 g/kg/day in children aged 1–10 y; and 1 g/kg/day in patients >10 years. This data was confirmed by a survey of 63 PKU centers from 18 countries, demonstrating that prescription patterns of total protein intake were influenced by country and location in Europe. In general, no more than 20% of energy
PKU in Turkey

Ahring et al. reported their opinion paper about dietary management of PKU in European countries that most centers preferred to use protein substitutes with protein equivalent content >30% by weight, with or without added carbohydrate or fat, and all preferred substitutes enriched with vitamins and minerals (Table IV). MacDonald et al. pointed out that both Phe tolerance and total protein requirement (i.e. natural protein+Phe-free amino acid= total protein intake) play an important role in the determination of dosage of Phe-free L-amino acids or protein substitution. It is a common practice that recommendations for the optimal amount of Phe-free amino acids are based on protein recommendations for healthy individuals. However, additional factors may influence protein utilization in PKU.

According to the literature, although no data supports a higher dose of Phe-free amino acid, an incremental compensation factor of 1.2 is being used in Dutch guidelines, which is parallel to the compensatory ratio of 20% in USA guidelines. This compensation is for losses due to indigestibility and protein quality for mainly vegetarian diets. The recommended calculation of L-amino acid requirement by EU guidelines is total protein intake (56 g/day) - natural protein intake (6 g/day) = 50 g/day. This is corrected with an additional 40% of L-amino acids from the protein substitute = 50 g/day × 1.4 = 70 g/day.

The Committee members have also emphasized that they frequently encountered inadequate protein intake especially during childhood, so clinicians should be cautious about protein restriction in a growing child (Table IV). A consensus has been reached that the amount of protein substitute according to age and severity of the disease should be determined first, and then the amount of dietary Phe intake should be decided. Studies have indicated that even when the total protein amount is increased they do not cause problems, unlike intact proteins, as there are L-amino acids in the substitutes and they are easily eliminated. Thus, a common decision has been reached that total protein amount should not be limited, and it should be calculated as 40% more than a healthy child at the same age, whereas at least 80% of the total amount should be provided by protein substitutes, which should be administered in divided doses (at least 3-4 doses in a day).

**Statement #8:**

- The total protein intake should not be limited and should supply age-related safe levels of protein intake (FAO/WHO/UNU 2007) with an additional 40% from L-amino acids supplements and a 20% compensatory factor to account for the digestibility and utilization of amino acids from the supplement, and a further 20% compensation to optimize Phe control.
- Phe-free amino acids should supply >75% of the total protein intake.
- Protein substitutes should be administered in divided doses (at least 3-4 doses in a day).

**Follow-up parameters and frequency**

Experts have discussed that PKU patients are followed-up under two main titles in Turkey:

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**Table IV:** Dosage of protein equivalent from protein substitutes (g/kg per day) reported by individual centers (dietary recommendations on PKU across European centers, 2009).

<table>
<thead>
<tr>
<th>Age</th>
<th>B</th>
<th>D</th>
<th>DK</th>
<th>E</th>
<th>I</th>
<th>N</th>
<th>NL</th>
<th>PL</th>
<th>TR</th>
<th>UK</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 y</td>
<td>≤2</td>
<td>2.0-2.3</td>
<td>2-3</td>
<td>3</td>
<td>Dependent on patient</td>
<td>2.5</td>
<td>2.5-2.0</td>
<td>2.4</td>
<td>2</td>
<td>3*</td>
</tr>
<tr>
<td>1-3 y</td>
<td>1.2</td>
<td>1.7</td>
<td>2</td>
<td>2.5</td>
<td>2.5-2.0</td>
<td>2.0-1.8</td>
<td>1.6</td>
<td>1.5</td>
<td>3*</td>
<td></td>
</tr>
<tr>
<td>4-10 y</td>
<td>1.2</td>
<td>1.4-1.6</td>
<td>2</td>
<td>2</td>
<td>2.0-1.5</td>
<td>1.5</td>
<td>1.6</td>
<td>1.5</td>
<td>2*</td>
<td></td>
</tr>
<tr>
<td>&gt;10 y</td>
<td>1.0</td>
<td>0.8-1.1</td>
<td>10-14 y: 1.5 &gt;14 y: 1</td>
<td>1.5</td>
<td>1.5-1.0</td>
<td>1.2-1.0</td>
<td>1.2</td>
<td>1.2</td>
<td>1-1.5*</td>
<td></td>
</tr>
</tbody>
</table>

*Total protein including protein exchanges.

Centers: B: Belgium, D: Germany, DK: Denmark, E: Spain, I: Italy, N: Norway, NL: the Netherlands, PL: Poland, TR: Turkey, UK: United Kingdom.
the first is dealing with problems related to biochemical pathways of PKU, and the second is the follow-up of blood Phe levels, other amino acid levels, and nutritional parameters. According to the literature, deficiencies of some micronutrients are more common in PKU patients. These are mainly vitamins A, C, and E, selenium, coenzyme Q10, vitamins B2, B6, and B12, and folates (which can increase homocysteine levels in the blood), iron, zinc, calcium, carnitine, long-chain polyunsaturated fatty acid (LCPUFAs) and vitamin D.

The most comprehensive PKU patient follow-up schedule was published in the EU guidelines in 2017 (Table V). Minimum requirements for the management and follow-up of PKU patients are given according to age groups in a wide range from an outpatient visit to age group-specific investigations.

In PKU, the main factors influencing bone density are calcium and vitamin D status, the quality of bone proteins, endocrine status (alkaline phosphatase and PTH associated with an increase of calciuria and C-terminal telopeptide), and genetic and environmental factors. Therefore, adequate calcium and vitamin D intake, regular physical activity, and optimization of natural protein intake are recommended in the EU guideline. Moreover, experts suggest that although there is no sound research data about follow-up by dual-energy X-ray absorptiometry (DXA), bone mineral density (BMD) of PKU patients should be followed up during late adolescence.

In Turkey, despite physical constraints, and the absence of adequate infrastructure for PKU patients, practically experts follow up pediatric patients every three to six months until puberty, and once or twice a year from then onwards except for unexpected conditions. However, the follow-up schedule of blood Phe levels is close to the “ideal follow-up frequency of blood Phe” mentioned in Table III.

Neurocognitive follow-up in PKU

Children with PKU, whose treatment is initiated during the early days of life, encounter generally better developmental milestones, and they attend normal schools. Although much literature on early and continuously-treated PAH deficiency reports IQ scores in the average range, pediatric data suggest that even under these circumstances, children with PKU have IQ scores that are six to nine points lower than their siblings and parents. It is recommended that considering different literature data on normal IQ scores but failing functional outcomes in some of the children with PKU, deficits in executive functions in these patients warrant special attention during the follow-up. When adult PKU patients are considered, both EU and USA guidelines recommend annual routine neurological examinations.

Committee members discussed achievements obtained by the Trust for Children Phenylketonuria and Other Metabolic Diseases (METVAK), such as performing different tests for neurocognitive examination under the supervision of Child and Adolescent Psychiatrists. While only IQ has been tested previously, cognitive functions are being evaluated currently. These improvements have clarified that even patients with normal IQ scores may have problems in executive functions. Therefore, IQ scores alone are not a reliable indicator for healthy upper cortical functions. On the other hand, it is underlined that the insufficiency of trained manpower, infrastructure, and tools for evaluation, such as validated tests are primary drawbacks in detailed data collection. Members have agreed that the first evaluation of neurocognitive functions should be performed at least 2 times before 3 years of age, and once before 6 years of age by using the Denver Developmental Screening Test. It is proposed that Wechsler Intelligence Scale for Children–IV (WISC-IV) should also be performed at the preschool age and during adolescence. They have recommended neurocognitive evaluation in
Table V. Minimum requirements for the management and follow-up of PKU patients (the European guidelines on PKU, 2017).  

<table>
<thead>
<tr>
<th></th>
<th>Childhood (&lt;12 y)</th>
<th>Adolescence (12-18 y)</th>
<th>Adulthood (≥18 y) excluding maternal PKU</th>
<th>Maternal PKU</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outpatient visit</strong></td>
<td>Given good clinical and metabolic control: Age 0–1 year: every 2 months Age 1–12 years: twice per year Extra clinic visits as indicated</td>
<td>Given good clinical and metabolic control: twice per year Extra clinic visits as indicated</td>
<td>Given good clinical and metabolic control: once per year Extra clinic visits as indicated</td>
<td>Given good clinical and metabolic control: once per trimester Extra clinic visits as indicated</td>
</tr>
<tr>
<td><strong>Clinical nutritional assessment</strong></td>
<td>Every outpatient visit: dietary assessment (3-day food record/24 h recall), anthropometric parameters (weight, height, BMI) and clinical features of micronutrient and Phe deficiency (especially anorexia, listlessness, alopecia, perineal rash)</td>
<td>Every outpatient visit: dietary assessment (3-day food record/24 h recall), anthropometric parameters (weight, height, BMI) and clinical features of micronutrient and Phe deficiency</td>
<td>Every 12–24 months: dietary assessment (3-day food record/24 h recall), anthropometric parameters (weight, height, BMI), and clinical features of micronutrient and Phe deficiency</td>
<td>Every outpatient visit dietary assessment (3-day food record/24 h recall) and weight</td>
</tr>
<tr>
<td><strong>Metabolic control</strong></td>
<td>Age 0–1 year: Phe weekly Age 1–12 years: fortnightly Phe Increased frequency as indicated Annually: plasma amino acids</td>
<td>Monthly Phe Increased frequency as indicated Annually: plasma amino acids</td>
<td>Monthly Phe Increased frequency as indicated Annually: plasma amino acids</td>
<td>Pre-conceptionally: weekly Pregnancy: weekly Increased frequency as indicated Pre-conceptionally: plasma amino acids</td>
</tr>
<tr>
<td><strong>Biochemical nutritional assessment</strong></td>
<td>Annual measurement of plasma homocysteine and/or methylmalonic acid, hemoglobin, MCV, and ferritin. All other micronutrients (vitamins and minerals including calcium, zinc, selenium) or hormones (parathyroid hormone) if clinically indicated</td>
<td></td>
<td>Pre-conception and at the start of pregnancy: folic acid, vitamin B12, plasma homocysteine and/or methylmalonic acid, ferritin, full blood count Pregnancy: when indicated</td>
<td></td>
</tr>
<tr>
<td>Table V. Continued.</td>
<td>Childhood (&lt;12 y)</td>
<td>Adolescence (12-18 y)</td>
<td>Adulthood (≥18 y) excluding maternal PKU</td>
<td>Maternal PKU</td>
</tr>
<tr>
<td>---------------------</td>
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</tr>
<tr>
<td>Bone density</td>
<td>BMD measurement only indicated when there are specific clinical reasons or when patients are known to be at a particular risk of metabolic bone disease</td>
<td>The first measurement of BMD should be undertaken during late adolescence. When BMD is abnormal, DXA (with or without change of treatment) should be repeated after 1 year. If osteoporosis (BMD &lt; -2.5 SD) persists despite optimization of diet and physical activity, other possible causes of osteoporosis should be investigated. Treatment (including consideration of bisphosphonates) should be determined by osteoporosis severity. If BMD results are still low but stable, yearly measurement is unnecessary. When BMD is normal, no-repeat measurement is necessary. Further study needs only be considered when there are clinical reasons to do so.</td>
<td>BMD measurement is only indicated when there are specific clinical reasons or when patients are known to be at particular risk of metabolic bone disease</td>
<td>Not indicated</td>
</tr>
<tr>
<td>Neurocognitive functions</td>
<td>Only neurocognitive tests when indicated.</td>
<td>Testing at age 12 years. Proposed domains of neurocognitive testing: IQ, perception/visuospatial functioning, EF (divided into inhibitory control, working memory, and cognitive flexibility), and motor control. Extra neurocognitive tests as indicated.</td>
<td>Testing at age 18 years. Proposed domains of neurocognitive testing: IQ, perception/visuospatial functioning, EF (divided into inhibitory control, working memory, and cognitive flexibility), and motor control. Extra neurocognitive tests as indicated.</td>
<td>Not indicated</td>
</tr>
<tr>
<td>Age group-specific investigations</td>
<td>Childhood (&lt;12 y)</td>
<td>Adolescence (12-18 y)</td>
<td>Adulthood (≥18 y) excluding maternal PKU</td>
<td>Maternal PKU</td>
</tr>
<tr>
<td>----------------------------------</td>
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<td>------------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Adaptive issues (e.g., Clinical relevant behavioral problems)</td>
<td>Annually: clinical assessment/discussion</td>
<td>Annually: clinical assessment/discussion Screening at age 12 years</td>
<td>Annually: clinical assessment/discussion Screening at age 18 years</td>
<td>Not indicated</td>
</tr>
<tr>
<td>Neurological complications</td>
<td>If neurodegeneration occurs</td>
<td>If neurodegeneration occurs</td>
<td>Annually: clinical examination</td>
<td>Not indicated</td>
</tr>
<tr>
<td>Psychosocial functioning and wellbeing and QoL</td>
<td>Annually: Clinical assessment/discussion Once during childhood: (PKU-) QOL questionnaire</td>
<td>Annually: Clinical assessment/discussion Once during adolescence: (PKU-) QOL questionnaire</td>
<td>Annually: Clinical assessment/discussion Once during adulthood: (PKU-) QOL questionnaire</td>
<td>Especially in case of not becoming pregnant, the patient may need support</td>
</tr>
<tr>
<td>Psychiatric examination</td>
<td>At the onset of symptoms of psychiatric disturbances</td>
<td>At the onset of symptoms of psychiatric disturbances</td>
<td>At the onset of symptoms of psychiatric disturbances</td>
<td>Not indicated</td>
</tr>
<tr>
<td>White matter abnormalities (MRI)</td>
<td>When there is an unexpected clinical course and/or unexpected neurological deficits</td>
<td>When there is an unexpected clinical course</td>
<td>When there is an unexpected clinical course</td>
<td>Not indicated</td>
</tr>
</tbody>
</table>

Ultrasound at 18-22 weeks of pregnancy with screening for organ development (especially if there is a lack of optimal metabolic control)

Echocardiogram in all infants who are conceived by women with either high blood Phe levels or poor maternal blood Phe control during pregnancy
the following years during adulthood. Treating physicians should consider more sophisticated tests such as Bayley Scales of Infant and Toddler Developmental during the assessment, if available. For further assessments, patients with PKU should be referred to a certified clinical psychologist, if possible. In centers without a certified clinical psychologist, treating pediatric metabolic diseases specialists should perform the Denver Developmental Screening Test every 6 months as a part of the follow-up plan.

**Brain MRI**

It is reported in the literature that MR neuroimaging is almost always abnormal in adults with blood Phe levels consistently above 800 μmol/L showing evidence of white matter changes. The correlation of such abnormalities to clinical manifestations has not been demonstrated in the majority of studies and adverse long-term consequences have yet to be proven.\(^3\)\(^,\)\(^4\) White matter (WM) variations in MRI are reported reversible in PKU patients. Studies are showing an improvement of WM abnormalities (3 to 6 months) after lowering of blood Phe levels. However, the blood Phe values determined at outpatient control visits may not always reflect the absolute values. Clinically it is experienced in some patients that they have high blood Phe values between control visits, and they perform a depletion diet sometimes before they attend the visits. Recent advances in neuroimaging techniques such as diffusion kurtosis imaging (DKI), diffusion-weighted imaging (DWI), and diffusion tensor imaging (DTI) have enabled clinicians to assess the WM integrity and subtle changes in brain parenchyma.\(^2\)\(^,\)\(^3\)\(^,\)\(^2\)\(^3\) It has been reported that these novel neuroimaging techniques are sensitive to cognitive impairment as well as decreased diet compliance of PKU patients, thus may provide a significant tool for close follow-up of the central nervous system.\(^3\)\(^,\)\(^2\)\(^,\)\(^2\)\(^3\)

Under the light of literature, committee members have agreed that MR imaging should be performed to evaluate myelinization once in childhood, adolescence, and adulthood if there is no additional problem. However, the limited number of experienced radiologists and MRI centers are noted as the main drawbacks of this issue.

**Statement #9:**

- Blood Phe levels should be measured as stated in Statement 7. Blood samples of PKU patients who cannot attend control visits may be obtained in the nearest healthcare unit and can be sent to a reference unit. PKU treatment should be individualized in the required conditions.
- BMD should be performed during early adolescence. If there is a problem, it should be repeated once a year.
- Neurocognitive functions should be first evaluated at the preschool age (at least 2 times before 3 years of age, and once before 6 years of age). They should be evaluated during adolescence, and if possible, during adulthood.
- IQ testing should be first performed at the preschool age, and then during adolescence. Counseling centers may be used for this purpose.
- During preschool years, Denver Developmental Screening Test is a practical tool for follow-up and should be performed every 6 months in these patients.

**Parameters for anthropometric measurements**

In PKU patients, frequent dietary manipulations are required to respond to normal growth rate, and lifestages, and avoid concurrent diseases and comorbidities. Several studies in the literature indicate that transient growth retardation is common in children treated for PKU, especially from birth to the age of 3–5 years.\(^2\)\(^4\)–\(^2\)\(^5\) On the other hand, Acosta et al.\(^2\)\(^7\) showed that PKU children undergoing nutrition management, aged from 2 to 13 years old, presented a normal linear growth. Moreover, they determined that many children were overweight according to mean body mass index Z-scores. Studies show that children with PKU might weigh
more than normal children. Scaglioni et al. reported that the rate of overweight at the age of 8 years was around 25%. Even if PKU children are routinely long-term monitored for dietary intake, there are data showing evidence of overweight in this population. Therefore, ongoing nutritional assessment of energy intake and quality of carbohydrates in PKU diet, physical activity, and body weight in PKU patients should be carefully monitored over the life cycle (Table V and VI). If a patient is not consuming an adequate amount of protein substitute distributed throughout the day, not only will plasma Phe concentration be elevated due to protein catabolism, but growth failure may occur because protein substitutes provide the majority of vitamins and minerals (Table VI).

The committee members discussed whether growth charts defined for Turkish children were still useful in the determination of obesity and malnutrition in children with PKU. Experts stated that these charts were being used to follow-up growth rate, whereas National Institutes of Health (NIH) standards were being used in some other centers. The main discussion points were switching to WHO criteria, which were more valuable for malnutrition, and enabled earlier diagnosis. Center for Disease Control and Prevention (CDC) curves used for obesity evaluation, and the absence of specific growth charts for children with PKU. It was decided that weight, height, body mass index, and height for age along with head circumference would be the most useful anthropometric criteria for the follow-up.

Statement #10:
• Height and weight for age should be the anthropometric criteria used for the follow-up.
• Head circumference measurements should be continued up to 3 years.
• Since the circumference of the upper arm, and skin thickness can vary according to individual measuring, they should not be accepted as criteria for growth follow-up.

**Maternal PKU (MPKU)**

The teratogenic effects of Phe on the developing fetus, termed MPKU syndrome, refers to the

<table>
<thead>
<tr>
<th>Age</th>
<th>Protein requirement</th>
<th>Minimum Phe requirement</th>
<th>Range of Phe intake</th>
<th>Energy [26] kcal/kg/day</th>
<th>Energy [26] kcal/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6 months</td>
<td>3-3.5</td>
<td>20-70 [26]</td>
<td>-</td>
<td>95-145</td>
<td></td>
</tr>
<tr>
<td>7-12 months</td>
<td>2.5-3 (1.31) [26]</td>
<td>10-35 [26]</td>
<td>-</td>
<td>80-135</td>
<td></td>
</tr>
<tr>
<td>1-3 years</td>
<td>2-3 (1.02)</td>
<td>NA</td>
<td>200-400</td>
<td>900-1800</td>
<td></td>
</tr>
<tr>
<td>4-6 years</td>
<td>2 (0.87)</td>
<td>13-20 [12]</td>
<td>210-450</td>
<td>1300-2300</td>
<td></td>
</tr>
<tr>
<td>7-10 years</td>
<td>2 (0.92)</td>
<td>13-20 [12]</td>
<td>220-500</td>
<td>1650-3300</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-14 years</td>
<td>2 (0.90)</td>
<td>NA</td>
<td>225-900</td>
<td>2000-3750</td>
<td></td>
</tr>
<tr>
<td>15-18 years</td>
<td>2 (0.87)</td>
<td>NA</td>
<td>295-1100</td>
<td>2100-3900</td>
<td></td>
</tr>
<tr>
<td>≥18 years</td>
<td>NA (0.84)</td>
<td>4.6-13.6 [11]</td>
<td>290-1200</td>
<td>2000-3300</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-14 years</td>
<td>2 (0.89)</td>
<td>NA</td>
<td>250-750</td>
<td>1500-3000</td>
<td></td>
</tr>
<tr>
<td>15-18 years</td>
<td>2 (0.84)</td>
<td>NA</td>
<td>230-700</td>
<td>1200-3000</td>
<td></td>
</tr>
<tr>
<td>≥18 years</td>
<td>NA (0.84)</td>
<td>4.6-13.6 [11]</td>
<td>220-700</td>
<td>1400-2500</td>
<td></td>
</tr>
</tbody>
</table>

NA= Sufficient evidence is not available for this age group.

Protein requirements for PKU are based on increased need with consumption of protein substitute, and values in parentheses reflect WHO safe level recommendations for the typical population.
physical and cognitive effects on the fetus of in utero exposure to elevated Phe levels including microcephaly, and poor fetal growth, congenital heart defects (CHD), non-familial facial features, and intellectual disability. Since the identification of MPKU syndrome, concerns have been raised regarding the degree to which inadequate maternal treatment could negate the positive societal and economic effects of early identification through NBS. According to the literature, approximately 65% of mothers with PKU have poorly controlled Phe before 8 weeks gestation. In the first published series of 71 pregnancies and 45 live births of 32 women with PKU from Turkey, it was reported that microcephaly, intellectual disability, and dysmorphic faces were more prevalent in the offspring of untreated than treated pregnancies with classical PKU (100% vs. 0%, 91% vs. 0%, and 73% vs. 23%). It was also noted that Phe levels were higher during weeks 6–14 than other periods of gestation in treated pregnancies, and the authors concluded that more frequent Phe measurements during the late first trimester are crucial to improve outcomes in treated pregnancies.

Since the maternal to fetal concentration gradient results in Phe levels 1.5 to 2 times greater in the fetus than in the maternal plasma, it is recommended that women achieve plasma Phe concentrations of 120 to 360 μmol/L before pregnancy, as critical development of the fetal central nervous system and heart occurs between 5 and 8 weeks’ gestation.

In line with the accumulated evidence, women with PKU are strongly recommended to start a Phe-restricted diet before conception. For MPKU, although minimal outpatient clinic visits of once during each trimester is recommended, more frequent follow-up and intense monitoring according to individualized needs and metabolic control are generally preferred in the daily practice (Table V). Metabolic control is based on weekly Phe blood spots pre-conception and during pregnancy. Women with PKU should receive routine obstetric care and should return to standard dietary or pharmacological treatment after delivery.

The committee members pointed out that the main issue in MPKU was increasing awareness of obstetricians. It was discussed that joint meetings might be held about the association of metabolic disease with pregnancy. It was emphasized that if PKU is diagnosed during pregnancy or a female with uncontrolled PKU gets pregnant, abortion should be considered. Therefore, activities in Turkey should be planned and performed under the leadership of the Child Nutrition and Metabolism Association. It was agreed that a national database should be developed shortly to collect health data on all critical issues such as the prevalence of pregnant women with PKU.

**Statement #11:**

- Pregnant women with PKU should be followed up once a week in the first trimester.
- They should be followed up fortnightly when the organogenesis takes place.
- The target Phe level is < 5 mg/dl.

**Alternative therapies**

**Glycomacropeptide (GMP)**

GMP is a protein derived from cheese whey that is naturally low in Phe and is rich in valine, isoleucine, and threonine. Short-term data from a small controlled study in older patients indicated that GMP caused lower fasting Phe concentrations and blood urea nitrogen compared with Phe-free L-amino acid supplements. There is also the suggestion that GMP lowers post-prandial concentrations of the appetite-stimulating hormone ghrelin and may help promote satiety.

As there is no experience with GMP in Turkey, there is a need to test its palatability, acceptability, and its control on blood Phe levels in Turkish PKU patients.
Large Neutral Amino Acids (LNAAAs)

The LNAAAs, Phe, tyrosine, tryptophan, and the branched-chain amino acids share the same amino acid transport system across the blood-brain barrier. Therefore, at high concentrations, Phe in the blood will compete with other LNAAAs for transport across the blood-brain barrier. LNAA supplementation has been shown to reduce cerebral Phe concentrations while an increase in plasma Phe levels was observed.\(^1\) LNAA treatment appears to have a beneficial effect on executive functioning, however this treatment is presumed to be suitable for adults who are not adhering to a low Phe diet.\(^1\)

Besides, these supplements are not suitable for children under the age of 8 years as well as during pregnancy. There has been little evidence to support their routine use in PKU. Moreover, ideal dose determination and safety still require further studies.\(^3\) Therefore, it should be the treating physician’s discretion to decide on when and to whom to initiate LNAA supplementations. The LNAA supplementations may be recommended starting from adolescence.

The committee members discussed that although there were very limited data on LNAA use in Turkey, the results were satisfactory so far, mainly showing improved cognitive functions. It was emphasized that patient selection was critical, and all of the clinical experience was collected from old patients with PKU who had low adherence to treatment. The most commonly faced problem was the patient’s expectation of an abrupt decrease in blood Phe levels. It was decided that the most objective criteria to determine the efficacy of LNAA treatment were cognitive tests.

Polyethylene glycol phenylalanine ammonia-lyase (PegPAL)

Until recently, there have been no pharmacological treatment options for patients who are non-responsive to sapropterin other than a low Phe diet.\(^38\) It is a known fact that control of Phe levels is difficult especially in adolescents and young adults due to incompatibility with diet therapy, decreased quality of life due to difficulty in coping with daily life, and nonattendance to follow-up visits among all patients with hyperphenylalaninemia.\(^39\)

A recently introduced enzyme substitution therapy Pegvaliase (rAvPAL-PEG) has obtained approval for PKU patients older than 18 years of age by the FDA in May 2018, and for patients older than 16 years of age by the European Medicines Agency (EMA) in May 2019.\(^40,41\) Therefore, treatment with PAL enzyme, which metabolizes Phe independently from PAH enzyme, has in use for PKU treatment.\(^42\) It not only is effective in decreasing blood Phe levels but also has manageable side effects.

During treatment, statistically significant improvements in blood Phe concentrations have been reported.\(^42\) Moreover, it is determined that patients have sustained reductions in blood Phe concentrations that reached guideline-recommended levels.\(^42\) Clinical study results have indicated that Phe levels are markedly reduced in patients who have responded to pegvaliase treatment, even reduced below target levels.\(^39,42\)

However, due to the long duration of dose adjustment and titration, injectable route of administration, potential side effects extending to the risk of anaphylaxis, and the higher cost than conventional diet therapy it is not commonly used.\(^38,42\) In many centers, the decision for pegvaliase treatment depends on the patient-based selection of health authorities, disease burden, and degree of compliance with the diet.\(^38,39,42,43\)

In Turkey, Pegvaliase (rAvPAL-PEG) is approved by the scientific council for usage but is not reimbursed by social security institutions, yet. Since it is not available in clinical practice, clinical experience is limited in efficacy, safety, and side effect profile. The committee members have concluded that they will re-evaluate this issue when there is enough data.
**Tetrahydrobiopterin (BH4)**

BH4, also known as sapropterin dihydrochloride is used to treat a subset of PKU patients. Patients with high residual activity of the PAH enzyme have a greater probability of BH4 response, but a minority of patients with classical PKU also may benefit from BH4 treatment. As efficacy and safety of BH4 have been shown in children <4 years of age BH4 got European approval for this age category.\(^3\)

In a retrospective Turkish study conducted on 44 children with PKU who were younger than 4 years of age (median age=3.8 years), it was reported that the median BH4 treatment period of 26.7 months (12-45 months) was safe and effective (Phe tolerance was significantly increased by a median of 2.26 folds from 47.5 mg/kg/day to 114 mg/kg/day).\(^4\) Authors from European centers report that BH4-responsiveness should be determined on an individual case basis. The degree of responsiveness will be characterized by the extent of improvement in biochemical control and an increase in natural protein intake. It is defined as ‘establishing an increase in natural protein tolerance of ≥100% with blood Phe concentrations remaining consistently within the target range’. It can also be defined by ‘improved’ metabolic control >75% of blood Phe levels and remaining within the target range without any decrease in natural protein intake associated with BH4 treatment’. Moreover, it is recommended that BH4 should only be prescribed in cases of proven BH4-responsiveness.\(^3\)

If a newborn is diagnosed with PKU during neonatal screening, and the blood Phe level is <20 mg/dl, then the neonate is tested with a single oral dose of BH4. Continuation of the treatment is decided according to the test result. In older children and/or patients with low blood Phe levels, the diet liberalization or Phe loading is performed to increase the Phe levels over 10 mg/dl as reported in the literature. After 72 hours, patients have hourly oral BH4 tests. Also in patients with mutations, if they are known to have a responsive mutation listed in the database, BH4 is initiated and the diet is liberalized according to blood Phe levels. Therefore, it may be noted that there are three protocols:

- Newborns with blood Phe levels <20 mg/dl: 20 mg/kg BH4 over 24 hours.
- Older children, previously untested and under treatment: Phe overload by diet or Phe supplementation followed by 20 mg/kg/day BH4 challenge for 72 hours.
- Patients with mutations included in the database and known to be responsive: Direct initiation of treatment and gradual liberalization of the diet (if the sibling is known to have a responsive mutation, treatment may be initiated directly, even though the mutation is not defined).

The committee members discussed the limited data in BH4 treatment in Turkey, and outcomes were controversial because the patient populations were small, and there was no national standardization for eligibility and criteria of monitoring for BH4 treatment. For the time being, individualization of BH4 according to the patient with PKU was the most commonly preferred clinical practice.

**Gene therapy**

Recently gene therapy for PKU has been reported as a promising approach for both protection from neurocognitive problems, and providing a normal diet without Phe restriction for PKU patients. Preclinical studies using adeno-associated virus (AAV)-mediated gene addition therapy in the \(Pah^{enu2/enu2}\) mouse model in classical PKU\(^{45,46}\), genetically engineered probiotics for PKU treatment (phenylalanine lyase gene from \(Anabaena variabilis\) (AvPAL) in the \(Pah^{enu2/enu2}\) mouse model)\(^{47}\), genetically engineered live bacteria (\(Escherichia coli\) Nissle)\(^{48,49}\), gene correction therapy using CRISPR-Cas-9 associated base editors\(^{50}\) have reported these investigational therapeutic approaches might be novel interventions for PKU. Gene correction therapy using CRISPR-
Cas-9 associated base editors have been shown to provide >20% of the normal activity of PAH activity in Pahenu2 mice. It has been shown that the correction of PAH enzymatic activity has been improved with time and is not associated with unwanted DNA changes in genomic regions with homology to the guide RNA utilized. In (AAV)-mediated gene addition, the corrected genes are demonstrated to pass to new liver cells and maintain enzymatic activity with hepatocyte proliferation.

An alternative way to provide PAH gene is to supply PAH mRNA enclosed within lipid nanoparticles and the liver takes it up leading the hepatocytes to produce the enzyme without initiation of the immune response. There are new Phase 1 and Phase 2 studies initiated to provide more effective therapeutic approaches in PKU by using live biotherapeutic products (LBPs) such as SYNB1618 in addition to Escherichia coli Nissle 1917 (EcN).

Statement #12:

• GMP is unavailable in Turkey, so there is no experience with this product.
• Selection criteria for LNAA may be advanced age, refusal of protein substitute intake, and hearing about the product from other patients with PKU.
• Current information about PegPAL indicates that it is too early to be used in pediatric patients, and there are many issues to be resolved before it is accepted as a safe and reliable treatment option.
• BH4 should be used in every suitable patient. Criteria for selecting eligible patients for BH4 treatment should be reviewed for Turkey, too.

Similar to many congenital metabolic diseases requiring a diet, PKU is not only a metabolic emergency but also a social one.

Early diagnosis and treatment initiation; determination and standardization of diagnostic and treatment thresholds to minimize errors, treatment modalities, and follow-up parameters according to the country’s conditions will be the utmost significant step to obtaining the best outcomes in PKU in the long term.

This Recommendations Paper is prepared as a training document on main issues in PKU follow-up. It may function as a recommendation for clinicians in troubling pitfalls during daily practice.

European and USA consensus data are combined with accumulated knowledge from experienced clinics in PKU in Turkey and discussed with the literature.

It is pointed out that PKU follow-up is a dynamic process with uncertainties and differences in clinical practice, all of which have been carefully considered. Nevertheless, frequent revisions will be necessary.

The authors believe that when the national patient database is completed, the present review will have significant contributions to the current literature.

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Author contribution

The authors confirm contribution to the paper as follows: Study concept and design, drafting the manuscript, critical revision of the manuscript for important intellectual content, administrative support, data input and interpretation: TC; drafting the manuscript, critical revision of the manuscript for important intellectual content, administrative support, data input and interpretation: HGÖ; data input and interpretation, drafting the manuscript: MÇ, NÖM, SS. All authors reviewed the results and approved the final version of the manuscript.
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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES


