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RESEARCH REPORT

Alkaptonuria Severity Score Index Revisited: Analysing the AKUSSI and Its Subcomponent Features

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Abstract *Background*: Alkaptonuria (AKU) is a rare disorder with no licensed treatment; nitisinone may reduce symptoms and progression. The All Alkaptonuria Severity Score Index (AKUSSI) measures disease severity in clinical, joint and spine domains, with 57 subcomponent feature scores. Our primary aim was to assess tools for validating scores such as the AKUSSI by detecting relationships between features both before and during nitisinone treatment.

Methods: AKUSSI measurements from nitisinone-treated patients visiting the National AKU Centre between 01-Jun-2012 and 31-May-2016 were analysed pre-treatment, at first treatment and annually to Year 3 post-treatment. Principal component analysis (PCA) and redundancy analysis assessed whether any AKUSSI features contributed little information to the overall score.

Results: 65 AKU patients were included: 17 with a pre-treatment AKUSSI measurement (10 later received nitisinone) and 48 with a first measurement at their first treatment visit. In PCA, the first four principal components (PC1–PC4) explained ≥50% of AKUSSI variance at all visits (54.1–87.3%). Some features regularly dominated their domain's PC1: ears, aortic sclerosis, and nasal/temporal eye scores (clinical), pain-related scores (joint) and cervical, lumbar and thoracic spine scores (spine). Only the right-hand/wrist score was consistently redundant. Right eye (nasal) and left ear scores were redundant pre-treatment, potentially correlating with other dominant clinical PC1 features.

Conclusions: PCA and redundancy analysis supported the AKUSSI as a robust AKU disease severity measure, although some AKUSSI features could be removed for simplicity. For small patient populations and rare diseases, PCA and redundancy analysis together can aid validation of disease severity metrics.

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Introduction

Alkaptonuria (AKU; OMIM # 203500), known as "black bone disease" or "black urine disease", is a rare genetic disorder with a prevalence between 1:100,000 and 1:250,000 worldwide (Zatkova 2011; Gallagher et al. 2013). Symptom onset typically occurs when a person is in their 20s or 30s, with early symptoms similar to osteoarthrosis, such as stiffness and pain. AKU is due to inactivating mutations in the homogentisate 1,2-dioxygenase gene (HGD) causing a buildup of homogentisic acid (HGA). HGA accumulation in tissues causes ochronosis, leading to bone and joint degradation, urine darkening and discolouration of eyes and ears (Phornphutkul et al. 2002).

There is no recommended AKU treatment. Patients follow advice on a low-protein diet and physical activity (Arnoux et al. 2015). Patients may be prescribed vitamin C; however, there is no evidence of effectiveness (Ranganath

et al. 2013). Joint replacement, heart valve replacement and spinal surgery are often required as the disease progresses (Ranganath et al. 2013). Nitisinone, a competitive inhibitor of 4-hydroxyphenylpyruvate dioxygenase, an enzyme involved in producing HGA, reduces HGA excretion and completely blocks ochronosis in AKU mice (Preston et al. 2014). Clinical trials have shown that nitisinone reduces plasma levels and urinary excretion of HGA by ≥90% in humans (Suwannarat et al. 2005; Ranganath et al. 2016). Nitisinone is not a licensed AKU treatment but since 2012 has been used off label in the NHS England National Alkaptonuria Centre (NAC), at the Royal Liverpool University Hospital.

The multisystemic nature of AKU, with both rheumatological and clinical problems, has historically made disease severity and treatment efficacy difficult to quantify. The All Alkaptonuria Severity Score Index (AKUSSI) was developed to measure AKU disease severity (Cox and Ranganath 2011). This specialised metric consists of three domain scores based on clinical, joint and spine-related AKU features.

The 15 clinical domain feature scores represent observable factors such as eye and ear pigmentation, in addition to patient history, such as previous fractures. The joint domain (30 feature scores) includes the number of joint replacements, and pain associated with certain joints. The spine domain (12 feature scores) relates to kyphosis, scoliosis or pain in spinal areas. Supplementary Table S1 provides further details on the AKUSSI feature scoring system.

However, some of these 57 feature scores may measure similar aspects of AKU and some scores may not accurately reflect disease progression, adding noise to the AKUSSI. This may misrepresent disease severity and affect the utility of the score in assessing treatment efficacy.

When considering rare diseases such as AKU, with a small patient population available for clinical trials, there is limited power to assess treatment efficacy and samples may not be representative of the patient population or disease progression. Thus, there is a need to determine suitable methods to assess the validity of rare disease severity scoring systems such as the AKUSSI to ensure that they reflect disease nature and progression.

The primary aim of this analysis was to establish whether any AKUSSI features were redundant and could be removed to create a more robust disease severity measure.

Methods

Patients

The NAC at the Royal Liverpool University Hospital offers a free service to patients with AKU in England and Scotland. Here, nitisinone can be prescribed off label, if the patient is willing to undergo safety and efficacy monitoring. The service is open to all other AKU patients, including international patients, for a fee. Patients aged ≥16 years, with documented AKU based on urine HGA, and treated with nitisinone at the NAC between 1st June 2012 and 31st May 2016 were included in this analysis.

Any visit prior to receiving nitisinone was defined as Pre-NAC. Year 0 was the baseline visit where patients first received nitisinone. After patients began nitisinone treatment, they had up to three annual visits in the study time period: Year 1, Year 2 and Year 3. The "NAC period" is from Year 0 to Year 3.

All procedures were performed and approved as part of the National AKU Service program and were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients before being included in the study.

Statistical Analysis

Each visit (Pre-NAC to Year 3) was considered separately in all analyses, as data from the same patient are correlated, and rates of disease progression may change over time according to nitisinone treatment duration.

All statistical analyses were performed using R (version 3.4.1) (R Core Team 2017).

Principal Component Analysis

Principal component analysis (PCA) explored if any AKUSSI domains (clinical, joint or spine) contained feature scores explaining most of that domain score's variation, potentially indicating features with a large influence over the score. This analysis also determined if any feature scores consistently explained minimal variation and hence did not greatly add value to the AKUSSI. PCA was performed for each visit separately and the results were assessed to determine consistency across visits.

PCA requires all features to be on a comparable scale. Therefore, feature scores were pre-assessed for each domain at each visit; if variances of the feature scores differed substantially, features were standardised to have unit variance.

For each AKUSSI domain, PCA was implemented using R's "prcomp" function, which calculated linear combinations ("principal components") of the (standardised) feature scores. The exact composition highlights which features add the most value to the AKUSSI. The first principal component explains the most variation; the following principal components explain descending amounts. Each

principal component contains weights associated with each feature score, representing the proportion of that principal component described by each (standardised) feature.

In the presence of correlated variables, the first principal components can contain most of the data variation; thus, remaining principal components may be ignored without losing much information.

Feature scores with large weights are important for modelling variation in the AKUSSI. Any features with consistently low weights, or with higher weights only in later principal components, would suggest that these feature scores do not greatly contribute to the AKUSSI.

Redundancy Analysis

The aim of this analysis was to determine whether PCA results could be supported by an alternative analysis with more readily interpretable results. Redundancy analysis explored how effectively each feature could be predicted from the remaining features per AKUSSI domain. Redundancy was assessed between features sharing a domain, and was also assessed between the total scores of the clinical, joint and spine domains.

This redundancy analysis was performed using a stepwise approach, where each feature was individually predicted in turn, using R's "redun" function in the "Hmisc" package (Harrell 2014).

A feature was considered redundant if it could be predicted effectively by other features in its domain, using the coefficient of determination (R^2). R^2 is a measure of how much variability in the data can be explained by each feature in the stepwise redundancy model, with a higher R^2 indicating a better model fit to the data. However, standard R^2 will always increase for models with a larger number of variables. An alternative, adjusted R^2 method was therefore preferred, as this only increases in value if a new variable improves the model more than expected by chance. However, adjusted R^2 requires more patients than features. Thus, standard R^2 was used to assess the joint feature scores at the Pre-NAC and Year 3 visits.

Feature redundancy was statistically defined as a standard or adjusted R^2 value of ≥ 0.9; this conservative R^2 threshold was chosen to ensure that features identified as redundant were well-predicted by other features. Features with a very low R^2 or adjusted R^2 score (<0.1) were also investigated, as these correspond to features that were very difficult to predict. Such features may be key factors which describe a patient's AKU progression.

Results

Patient Disposition and Baseline Characteristics

A summary of the data provided by the NAC at the Royal Liverpool University Hospital is given in the patient flow diagram and baseline characteristics presented in Fig. 1.

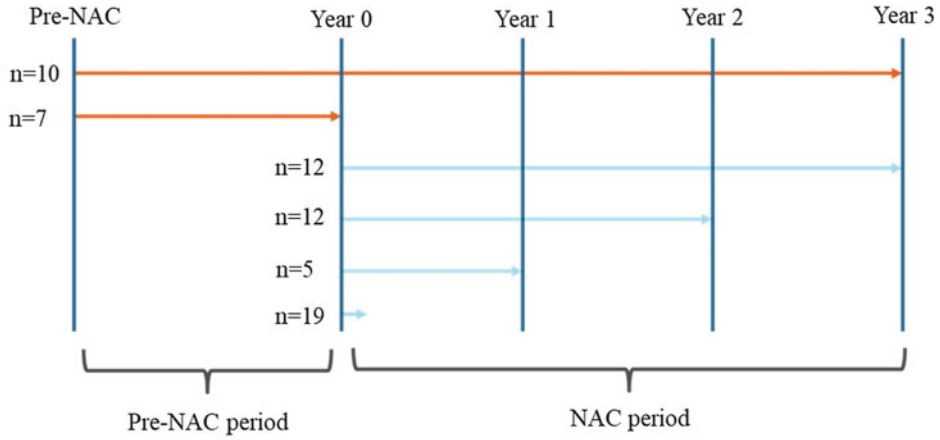
Sixty-five patients with AKU were included in the analysis. Seventeen patients' AKUSSI was measured before treatment with nitisinone began, at Pre-NAC. Of these, ten patients proceeded to receive treatment with nitisinone. Additionally, 48 patients began nitisinone treatment immediately after their first baseline (Year 0) visit. Patients followed a low-protein diet, which was managed by a dietician, to control tyrosinaemia. There were six dropouts: one death due to toxic megacolon, two patients having difficulty with the post-nitisinone low-protein diet, one patient unable to travel and two patients lost to follow-up (including one patient who did not return to the NAC following a stomach cancer diagnosis). All six of these patients had usable baseline (Year 0) data; two of these also had Pre-NAC AKUSSI scores recorded.

Principal Component Analysis

In each domain at each visit, the cumulative sum of the first four principal components explained between 54.1% and 87.3% of all variance. In all cases, the percentage of variance explained by the first principal component was approximately twice or more than that of the second component (Fig. 2).

In the clinical domain, the first principal component accounted for 34.8–41.0% of the variance for each visit; all other principal components at all visits accounted for less than 16% of the domain's variance (Fig. 2A). The first principal component was consistently dominated by the left and right eye features (both nasal and temporal) at all visits, and also left ear, right ear and aortic sclerosis features at all visits except Year 3, where the first principal component included ligament rupture at a similar level. No features consistently showed low contributions to PCA variation.

At each visit, one principal component of the clinical domain was dominated by kidney stones, and each of these principal components accounted for 7.9–15.7% of the visit's AKUSSI score variance. The tendon rupture feature also dominated a principal component at most visits; its principal component accounted for 10.4–12.3% of the variance and all were dominated by the tendon feature,



	Pre-NAC (n=17)*	Year 0 (n=65)†	Year 1 (n=39)	Year 2 (n=34)	Year 3 (n=22)
Characteristics, median [IQR] unless otherwise stated					
Age at visit, mean (SD)	47.8 (12.9)	47.4 (14.1)	47.3 (14.6)	48.7 (15.1)	47.3 (16.1)
Gender, female, n (%)	7 (41.2)	24 (36.9)	15 (38.5)	14 (41.2)	9 (40.9)
AKUSSI	79.0 [39.0, 104.0]	82.0 [45.0, 104.0]	81.0 [53.0, 104.5]	86.5 [59.0, 103.8]	83.0 [53.8, 104.5]
Joint Score	22.0 [12.0, 28.0]	22.0 [13.0, 32.0]	24.0 [14.5, 29.0]	26.0 [16.3, 32.0]	29.0 [15.5, 33.0]
Clinical Score	31.0 [16.0, 43.0]	28.0 [12.0, 43.0]	27.0 [14.0, 39.0]	29.5 [14.5, 40.8]	27.5 [18.5, 35.0]
Spine Score	20.0 [10.0, 32.0]	26.0 [14.0, 32.0]	28.0 [19.0, 34.0]	31.0 [21.0, 35.5]	30.0 [14.0, 34.0]
Measurements, mean (SE)					
Serum HGA, µmol/L	NR	27.3 (1.9)	5.4 (0.9)	4.7 (0.8)	4.6 (1.3)
Urine HGA, µmol/L/day	NR	22219 (1401)	2432 (1062)	1869 (729)	1646 (691)
Serum Tyrosine, µmol/L	NR	67.7 (10.6)	700.0 (26.3)	662.0 (38.4)	900.0 (24.9)

Fig. 1 Patient flow diagram and baseline characteristics. *Three patients had multiple Pre-NAC measurements; only their initial Pre-NAC visit was included in this analysis. †$n = 57$ patients had Year 0 HGA and tyrosine measurements. *AKU* alkaptonuria, *AKUSSI* AKU severity score index, *IQR* interquartile range, *HGA* homogentisic acid, *NAC* National Alkaptonuria Centre, *NR* not reported, *SD* standard deviation, *SE* standard error

occasionally combined with muscle rupture or hearing impairment features. All Year 3 patients had tendon scores of 0, thus no variance was associated with this feature at Year 3.

In the joint domain, the first principal component accounted for 25.7–29.2% of the variance at each visit; all other components at all visits accounted for less than 17% of the domain's variance (Fig. 2B). The first principal component was frequently dominated by pain scores, particularly foot and shoulder pain. The right foot and left foot scores in Year 2 and Year 3 were much less prominent across all components than they were for Pre-NAC, Year 0 and Year 1.

In the spine domain, the first principal component accounted for 42.8–57.2% of the domain's variance across visits; all other components at all visits accounted for less than 18% of the domain's variance (Fig. 2C). The first principal component was dominated almost entirely by cervical, lumbar and thoracic spine scores. In contrast, pain scores related to cervical, thoracic and lumbar spine areas only minimally contributed to any principal component variance at any visit. Variance in the kyphosis score is visible prior to nitisinone treatment; however, it becomes more prominent post-treatment, dominating the Year 1–3 second principal components. Scoliosis provided a minimal contribution to principal component variances at pre-nitisinone

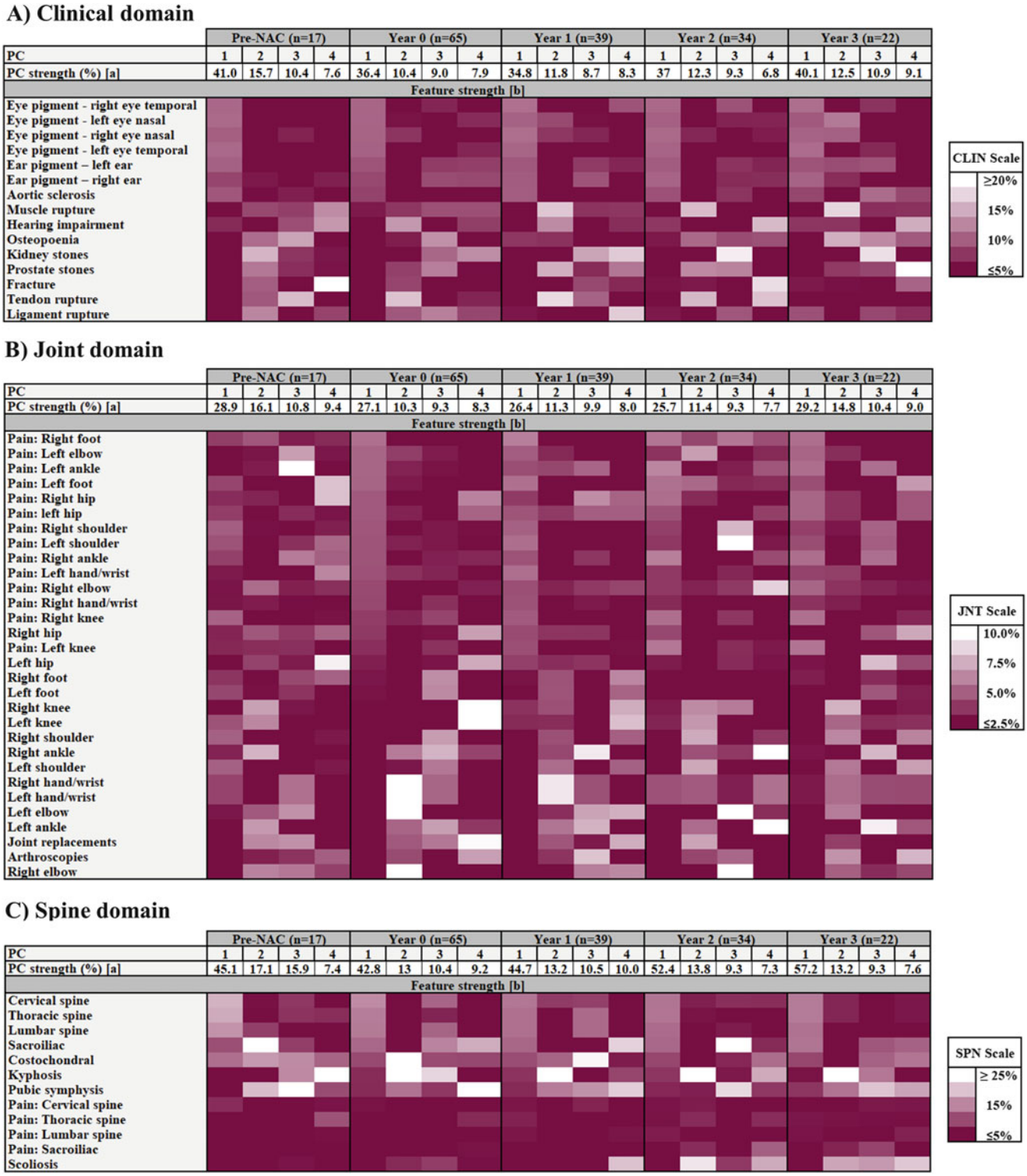


Fig. 2 Feature prominence in PCA for the A) clinical domain, B) joint domain and C) spine domain. [**a**] Proportion of the variance of the data at that visit explained by the given principal component. [**b**] Proportion of the individual principal component's variance explained by the given feature. Lighter rectangles denote a larger proportion of the variance; darker rectangles are features which do not contribute much to the variance; there is little difference between patients. *CLIN* clinical, *JNT* joint, *PC* principal component, *PCA* principal component analysis, *SPN* spine

visits, again becoming more prominent in principal components post-treatment.

Redundancy Analysis

No redundancy was identified between the total clinical, joint and spine scores for any visit. Individual domain features that were found to be redundant at $\geq$1 visit are presented in Table 1. A list of features not redundant at any visit, and those not well-predicted by other feature scores ([adjusted] $R^2 < 0.1$) for at least one visit, are shown in Table 2.

The right eye (nasal) and left ear features of the clinical domain were redundant at the Pre-NAC visit. However, no clinical domain features were redundant at subsequent treatment visits. Osteopenia, tendon rupture and muscle rupture were the least well predicted clinical score features across all visits.

Joint score features had high R^2 values across all visits, although only the right-hand/wrist score was consistently redundant. R^2 values were 1.0 for all joint scores at Pre-NAC and Year 3. These domain visits were analysed using ordinary R^2 rather than adjusted R^2 due to having fewer patient numbers than domain subcomponents; this large number of subcomponents will have increased the ordinary R^2 as discussed in the methods.

Cervical and thoracic spine scores were redundant at one and two visits, respectively. No other spine score features were redundant. Kyphosis and scoliosis features of the spine score had consistently low adjusted R^2 values across all visits.

Discussion

Both PCA and redundancy analysis supported the AKUSSI as a measure of AKU disease severity, as the majority of

Table 1 Redundant AKUSSI features and corresponding adjusted R^2 values

Feature	Pre-NAC[a] ($n = 17$)	Year 0 ($n = 65$)	Year 1 ($n = 39$)	Year 2 ($n = 34$)	Year 3[a] ($n = 22$)
Clinical score features (adjusted R^2)					
Eye pigment – right eye nasal	**0.93**	0.78	0.82	0.82	0.64
Ear pigment – left ear	**0.95**	0.70	0.59	0.70	0.89
Joint score features ([adjusted] R^2)					
Pain: right hip	1.00	0.76	0.69	0.42	**1.00**
Pain: left hip	1.00	0.70	0.79	0.42	**1.00**
Pain: right knee	**1.00**	0.65	0.43	0.70	1.00
Pain: right ankle	1.00	0.77	0.63	0.86	**1.00**
Pain: left ankle	**1.00**	0.86	0.69	**0.95**	1.00
Pain: right foot	**1.00**	0.80	0.74	**0.98**	1.00
Pain: left foot	**1.00**	0.77	0.79	0.87	**1.00**
Pain: left shoulder	**1.00**	0.75	**0.91**	0.89	1.00
Pain: left elbow	**1.00**	0.76	0.70	0.85	**1.00**
Pain: right hand/wrist	1.00	0.64	0.88	0.77	**1.00**
Left hip	**1.00**	0.68	0.50	0.65	1.00
Right knee	**1.00**	0.73	0.71	0.38	1.00
Right ankle	**1.00**	0.86	**0.96**	0.98	1.00
Right foot	**1.00**	0.52	0.00	0.00	**1.00**
Left knee	**1.00**	0.72	0.45	0.33	1.00
Right shoulder	**1.00**	0.50	0.23	0.00	1.00
Right elbow	**1.00**	0.71	0.89	0.61	1.00
Left elbow	1.00	0.75	**0.92**	0.52	1.00
Right hand/wrist	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
Left hand/wrist	1.00	1.00	1.00	1.00	**1.00**
Spine score features (adjusted R^2)					
Cervical spine	0.76	0.74	0.71	0.86	**1.00**
Thoracic spine	0.67	0.77	**1.00**	**0.93**	1.00

Features highlighted in **bold** were found to be redundant ([adjusted] $R^2 \geq 0.9$) at the relevant visit and removed from the analysis
[a] Ordinary R^2 were calculated for feature scores in the joint domain at these visits due to limited patient numbers

Table 2 Non-redundant AKUSSI features and corresponding adjusted R^2 values

Feature	Pre-NAC[a] ($n = 17$)	Year 0 ($n = 65$)	Year 1 ($n = 39$)	Year 2 ($n = 34$)	Year 3[a] ($n = 22$)
Clinical score features (adjusted R^2)					
Eye pigment – right eye temporal	0.92	0.79	0.87	0.82	0.81
Eye pigment – left eye temporal	0.87	0.76	0.84	0.63	0.78
Ear pigment – right ear	0.88	0.61	0.72	0.76	0.89
Eye pigment – left eye nasal	0.94	0.79	0.70	0.72	0.72
Prostate stones	0.40	**0.08**	**0.00**	**0.00**	**0.00**
Kidney stones	0.73	**0.01**	**0.00**	**0.00**	**0.00**
Osteopenia	**0.00**	**0.02**	0.20	**0.08**	**0.00**
Fracture	**0.00**	**0.00**	**0.00**	**0.00**	0.60
Tendon rupture	0.32	0.21	0.13	**0.00**	NA
Muscle rupture	**0.08**	0.24	**0.00**	**0.01**	**0.02**
Ligament rupture	**0.04**	**0.03**	0.25	**0.08**	0.73
Aortic sclerosis	0.64	0.36	0.41	0.49	0.11
Hearing impairment	**0.00**	0.25	0.27	0.28	0.40
Joint score features ([adjusted] R^2)					
Pain: left knee	1.00	0.61	0.49	0.58	1.00
Pain: right shoulder	1.00	0.76	0.88	0.86	1.00
Pain: left hand/wrist	1.00	0.68	0.86	0.80	1.00
Pain: right elbow	1.00	0.43	0.75	0.79	1.00
Right hip	1.00	0.75	0.66	0.81	1.00
Right foot	1.00	0.52	**0.00**	**0.00**	1.00
Left foot	1.00	0.65	0.30	NA	1.00
Left ankle	1.00	0.83	0.96	0.98	1.00
Right shoulder	1.00	0.50	0.23	**0.00**	1.00
Left shoulder	1.00	0.25	0.26	0.29	1.00
Number of arthroscopies	1.00	0.20	0.71	0.70	1.00
Number of joint replacements	1.00	0.46	0.20	**0.00**	1.00
Spine score features (adjusted R^2)					
Pain: cervical spine	0.20	0.49	0.61	0.64	0.76
Pain: thoracic spine	**0.00**	0.54	0.72	0.47	0.69
Pain: lumbar spine	**0.00**	0.28	0.49	0.78	0.74
Pain: sacroiliac	**0.00**	0.38	0.28	0.30	0.15
Lumbar spine	0.30	0.74	1.00	0.84	0.75
Sacroiliac	0.31	0.25	0.25	0.31	0.60
Pubic symphysis	0.31	**0.06**	0.11	**0.09**	**0.08**
Costochondral	**0.00**	0.24	0.15	0.47	0.34
Kyphosis	**0.00**	**0.07**	**0.00**	**0.02**	**0.00**
Scoliosis	**0.00**	**0.00**	**0.00**	**0.03**	**0.00**

Features listed in this table were those not found to be redundant ([adjusted] $R^2 \geq 0.9$) at any visit, and/or those not well-explained by other features scores ([adjusted] $R^2 < 0.1$) for at least one visit. Features highlighted in **bold** were those with [adjusted] $R^2 < 0.1$ for at least one visit

[a] Ordinary R^2 were calculated for feature scores in the joint domain at these visits due to limited patient numbers

feature scores were necessary to explain the variance in data from patients both before and during nitisinone treatment. In PCA, a large amount of AKUSSI information was held in feature scores in the first principal component, but later components were still valuable.

For the clinical domain PCA, the first principal component was consistently dominated by all eye and ear feature scores at all visits, showing potential relationships between these features. In the redundancy analysis, the right eye (nasal) and left ear feature scores were redundant at the

Pre-NAC visit, but not at later visits; however, R^2 values were high at all visits. This consistency between PCA and redundancy analysis may suggest that some of these feature scores are correlated, and potentially redundant. This could motivate removing the right eye (nasal) and left ear feature scores from this domain to simplify the score while losing minimal information, particularly as these feature scores do not contribute much to the principal components 2–4 at most visits.

In the joint domain, the pain-related feature scores consistently dominated the first principal component across all visits. Many of these were redundant at two separate visits in the redundancy analysis, such as the right/left foot pain, left shoulder pain and left ankle pain. Similar to the clinical domain, this suggests that these features, those that both compose the first principal component while also being redundant or at risk of redundancy (high R^2), may be correlated. The right ankle joint domain score was also redundant at two separate visits, and the right-hand/wrist feature was redundant at all visits. In the PCA, the right-hand/wrist often forms a principal component with the left-hand/wrist, left ankle and joint replacements, suggesting that these features may be related; this, in combination with the redundancy analysis, may suggest that right hand/wrist is related to these feature scores and could be removed from the AKUSSI.

In the spine domain, the first principal component's largest contributors were the cervical, thoracic and lumbar spine scores. In redundancy analysis, the cervical spine R^2 was high at all visits, and redundant at Year 3. Similarly, the thoracic spine score R^2 was above 0.66 at all visits, and redundant for Years 1–2. Results of PCA and redundancy analysis together may suggest that cervical and thoracic spine scores are correlated with the lumbar spine score, with only the latter required to explain much of the variance in the AKUSSI. All pain-related spine features showed minimal contribution to any principal component at any visit. The R^2 of the pain-related feature scores in the redundancy analysis were variable, with no clear pattern across the visits. Therefore, while redundancy analysis results suggest that pain-related spine scores are not redundant, PCA results could motivate removing them from the AKUSSI without losing much information. Both the kyphosis and scoliosis features were strong aspects of one principal component across the post-nitisinone visits. Kyphosis is also often a dominant feature in pre-nitisinone visits, although not as strongly. In redundancy analysis, both features have consistently low R^2 across all visits (0.00–0.07). Both analysis methods highlight the value of these feature scores in the AKUSSI.

Pigment building up in intervertebral discs and articular cartilage in vertebrae is thought to make discs increasingly rigid and fixed, such that body weight and muscle dominance change spinal curvature (Phornphutkul et al. 2002). If nitisinone minimises pigment buildup by decreasing HGA, kyphosis and scoliosis progression should also be slowed. Thus, one interpretation of the results of this analysis is that the increased AKUSSI variance explained by the kyphosis and scoliosis post-nitisinone treatment could reflect these potentially decreasing rates of disease progression. However, as kyphosis and scoliosis also depend on factors such as individual posture and physical activity (Kado 2009), effects of nitisinone could still be variable between patients.

Alternative methods for determining AKU disease severity include urine HGA measurements, which have investigated the effectiveness of nitisinone in AKU patients (Ranganath et al. 2016). However, a previous study has shown that where urine HGA concentrations were significantly reduced, clinical parameters were unchanged (Introne et al. 2011), suggesting that HGA levels alone may not be sufficient to measure disease severity.

For both PCA and redundancy analysis, an important limitation is that the number of patients may not have been sufficiently large to accurately determine feature score variability; this is common in rare diseases, and any trends should be interpreted with care. Due to the limited number of patients, each domain was analysed separately. Therefore, it was not possible to identify relationships between features of different domains. In addition, where standard R^2 was required, results should be interpreted with caution, as discussed in the methods.

Analyses assumed that relationships between features were linear. Non-linear combinations may explain more variation but were not considered due to the difficulty of interpretation. While PCA with linear models is commonly used, such outputs can be hard to interpret alone. By comparing the consistency of both PCA and redundancy analysis, this provides a more complete picture of the AKUSSI, its domains and their features. This dual approach is valuable in rare conditions such as AKU, and other situations where only a limited number of patients can be assessed.

While the majority of patients included in these analyses were from the UK, a minority were from other countries. Therefore, results of these analyses are predominantly relevant to UK-treated patients, and further research may determine generalisability to AKU patients worldwide.

Conclusion

Both PCA and redundancy analysis supported the AKUSSI as a measure of AKU disease severity, with most feature scores proving necessary to explain the AKUSSI variance before and during nitisinone treatment.

For small patient populations and rare diseases, PCA and redundancy analysis together can be valuable for validating disease severity metrics.

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Synopsis

Rare disease severity scores can be assessed using a combination of principal components analysis and redundancy analysis; using this method, the AKUSSI metric was shown to be robust in assessing alkaptonuria disease severity.

Author Contributions

All authors meet the International Committee of Medical Journal Editors' criteria for authorship and have made substantial contributions to the conception, design, execution or analysis and interpretation of the data:

> Study conception/design and acquisition of data: BL, AH, OT, JAG and LRR; analysis/interpretation of data: BL, MB, AH, LE, OT, JAG and LRR; drafting of the publication, or revising it critically for important intellectual content: BL, MB, AH, LE, OT, JAG and LRR; final approval of the publication: BL, MB, AH, LE, OT, JAG and LRR.

The authors confirm independence from the sponsors; the content of the article has not been influenced by the sponsors.

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Competing Interest Statement

B. Langford, M. Besford, A. Hall and L. Eddowes are employees of Costello Medical, Cambridge, UK. L. Ranganath, J. A. Gallagher and O. Timmis work on the DevelopAKUre project investigating the use of nitisinone as a potential treatment for AKU patients. O. Timmis was the CEO of the AKU society, Cambridge, UK while contributing to this research. Editorial support was provided by Costello Medical.

Funding

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Ethics Approval

All procedures followed were performed and approved as part of the National AKU Service program and were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

Patient Consent

Informed consent was obtained from all patients for being included in the study.

References

Arnoux JB, Le Quan Sang KH, Brassier A et al (2015) Old treatments for new insights and strategies: proposed management in adults and children with alkaptonuria. J Inherit Metab Dis 38 (5):791–796

Cox TF, Ranganath L (2011) A quantitative assessment of alkaptonuria: testing the reliability of two disease severity scoring systems. J Inherit Metab Dis 34(6):1153–1162

Gallagher JA, Ranganath LR, Zatkova A (2013) Alkaptonuria. In: Brenner's encyclopedia of genetics, 2nd edn. Academic Press, Cambridge, pp 71–75

Harrell FE (2014) Hmisc: a package of miscellaneous R functions. http://biostat.mc.vanderbilt.edu/Hmisc

Introne WJ, Perry MB, Troendle J et al (2011) A 3-year randomized therapeutic trial of nitisinone in alkaptonuria. Mol Genet Metab 103(4):307–314

Kado DM (2009) The rehabilitation of hyperkyphotic posture in the elderly. Eur J Phys Rehabil Med 45(4):583–593

Phornphutkul C, Introne WJ, Perry MB et al (2002) Natural history of alkaptonuria. N Engl J Med 347(26):2111–2121

Preston AJ, Keenan CM, Sutherland H et al (2014) Ochronotic osteoarthropathy in a mouse model of alkaptonuria, and its inhibition by nitisinone. Ann Rheum Dis 73(1):284–289

R Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/

Ranganath LR, Jarvis JC, Gallagher JA (2013) Recent advances in management of alkaptonuria (invited review; best practice article). J Clin Pathol 66(5):367–373

Ranganath LR, Milan AM, Hughes AT et al (2016) Suitability Of Nitisinone In Alkaptonuria 1 (SONIA 1): an international, multicentre, randomised, open-label, no-treatment controlled, parallel-group, dose-response study to investigate the effect of once daily nitisinone on 24-h urinary homogentisic acid excretion in patients with alkaptonuria after 4 weeks of treatment. Ann Rheum Dis 75(2):362–367

Suwannarat P, O'Brien K, Perry MB et al (2005) Use of nitisinone in patients with alkaptonuria. Metabolism 54(6):719–728

Zatkova A (2011) An update on molecular genetics of Alkaptonuria (AKU). J Inherit Metab Dis 34(6):1127–1136