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Clinical features and amino acid profiles of dogs with hepatocutaneous syndrome or hepatocutaneous-associated hepatopathy

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Abstract

Background: Superficial necrolytic dermatitis (SND), hepatocutaneous-associated hepatopathy (HCH), aminoaciduria, and hypoaminoacidemia define hepatocutaneous syndrome (HCS) in dogs. Dogs without SND but that possess all other syndrome components are not well described.

Hypothesis/objectives: To define an inclusive syndrome, aminoaciduric canine hypoaminoacidemic hepatopathy syndrome (ACHES) for dogs with HCH or HCS. Compare clinical features, salient clinicopathologic variables, and plasma and urine amino acid (AA) profiles among ACHES cases by skin lesion status.

Animals: Dogs of various breeds and ages diagnosed with ACHES (n = 41). A control (CON) cohort (n = 12) provided AA profile data.

Methods: Retrospective case series. Available medical records of previously identified cases were reviewed for salient clinical features and clinical pathology data. Plasma and urine AA profiles were performed. Cutaneous lesion status was classified as none, mild, or fulminant.

Results: Thirty cases (73%) developed SND at some time. Dogs with fulminant skin lesions at diagnosis (n = 22/41, 54%) had significantly lower hematocrit ($P = .05$) and mean corpuscular volume ($P = .01$) than dogs without SND. Principal component analysis of plasma AA profiles identified distinct clustering of CON from ACHES dogs, but not by skin lesion status. Plasma 1-methylhistidine (<7 nmol/mL) and cystathionine (<7.5 nmol/mL) were robust ACHES biomarkers. Urine lysine (>344 nmol/mg creatinine) and methionine (>68 nmol/mg creatinine) also were useful ACHES biomarkers.

Conclusions and Clinical Importance: Specific AA biomarkers provide additional diagnostic utility in ACHES. Data suggests that HCH is an early stage, and SND a later stage manifestation of ACHES.

Abbreviations: AA, amino acid; ACHES, aminoaciduric canine hypoaminoacidemic hepatopathy syndrome; ALP, alkaline phosphatase; ALT, alanine aminotransferase; DM, diabetes mellitus; HCS, hepatocutaneous syndrome; HCH, hepatocutaneous-associated hepatopathy; HCT, hematocrit; MCV, mean corpuscular volume; PC, principal component; PCA, principal component analysis; ROC, receiver-operating characteristics; SND, superficial necrolytic dermatitis.

KEYWORDS
hepatic disease, internal medicine-canine, metabolic disease

1 | INTRODUCTION

For decades, cutaneous lesions have eponymously defined canine hepatocutaneous syndrome (HCS). The skin lesions of HCS, superficial necrolytic dermatitis (SND), were first reported in humans as a condition associated with glucagonomas in 1942.[1] Since first being described as a diabetic dermatopathy in 1986,[2] our understanding of SND in dogs has evolved considerably. Descriptions of dogs with SND identified a common association with a unique hepatocutaneous-associated hepatopathy (HCH), thus, the term HCS was applied. Later studies identified hypoaminoacidemia and aminoaciduria as key elements of the syndrome.[3-10] However, dogs with HCH, hypoaminoacidemia, and aminoaciduria but lacking SND lesions at initial diagnosis, and speculated to represent a later stage syndromic manifestation, have been encountered.[3,5,9-13] Because HCS implies the presence of SND lesions, we propose an alternative term: aminoaciduric canine hypoaminoacidemic hepatopathy syndrome (ACHES) to include all dogs with the metabolic syndrome of HCH, hypoaminoacidemia, and aminoaciduria, regardless of the presence or absence skin lesion status.

Proposing this new diagnostic acronym requires validating ACHES as a syndrome with diagnostic criteria inclusive of cases with or without SND (ie, ACHES-SND or ACHES-CLF [cutaneous lesion free]). Thus far, no known reports compare the clinical features, clinical pathology, or amino acid (AA) profiles of dogs with or without skin lesions. The histologic features of HCH, including severe vacuolar hepatopathy, parenchymal collapse, and proliferative nodules, often are identified on ultrasound evaluation of the liver.[3,5,6,10] Hypoaminoacidemia is a widely reported and consistent feature of SND, whereas aminoaciduria was described more recently.[5,9,13] Although dogs diagnosed with HCH lacking skin lesions are hypoaminoacidemic and aminoaciduric, their AA profile results were not analyzed separately from or compared to dogs with SND.[5] Comparing salient features between cases with (ACHES-SND) or without (ACHES-CLF) SND is a first step in corroborating the speculation that HCH and hypoaminoacidemia precede SND in HCS or ACHES. If this sequential pathogenesis is correct, early diagnosis of ACHES by recognizing HCH may substantially improve the prognosis in this typically treatment-resistant disorder.

Our objectives were to compare: (a) clinical features, including SND lesions, diabetes mellitus (DM), and hepatic ultrasound examination findings; (b) salient clinical pathology results of hematocrit (HCT), mean corpuscular volume (MCV), alkaline phosphatase activity (ALP), alanine aminotransferase activity (ALT), serum albumin and cholesterol concentrations; and (c) plasma and urine AA profile results among ACHES cases with different skin lesion status.

2 | MATERIALS AND METHODS

2.1 | Case selection criteria

One investigator (JPL) identified all enrollment cases from enrollment in ongoing studies investigating ACHES between 2014 and 2019. Diagnostic criteria similar to those previously described were applied.[5] A histologic diagnosis of ACHES required SND or HCH lesions present in skin or liver biopsy specimens, respectively, as previously described.[3,10] A board-certified pathologist (JPK) provided histologic diagnoses. Additionally, a board-certified internist with expertise in hepatic pathology (SAC) recruited cases with HCH lesions, and reviewed all liver histology. A clinical diagnosis of ACHES without a histologic diagnosis required the following findings consistent with HCS/ACHES: (a) macroscopic appearance and distribution of skin lesions, (b) compatible clinical pathology findings such as mild to markedly increased ALP activity, abnormalities in other hepatic enzymes, microcytosis or some combination of these, (c) classic so-called Swiss cheese appearance of nodular liver parenchyma on ultrasound examination, and (d) confirmatory results of plasma and urine AA profiles were required for all cases without a histologic diagnosis. Control (CON) dogs were chosen based on unremarkable history (except for DM, or mild abnormalities of liver enzymes, which were both permitted for CON), physical examination findings, clinical pathology results, and plasma AA profile results. All AA profiles were performed by a reference veterinary laboratory (Amino Acid Laboratory, College of Veterinary Medicine, University of California-Davis, Davis, California) as previously described near the time of ACHES diagnosis.[5] Urine AA concentrations were normalized to urine creatinine concentrations. Exclusion criteria included: (a) glucagonoma, (b) failure to develop skin lesions in cases without a histologic diagnosis, or (c) skin or liver histopathology results inconsistent with SND or HCH. The Institutional Animal Care and Use Committee of Cornell University approved animal use for this study.

2.2 | Data collection

We sent an electronic survey (Supplementary File 1) to veterinarians or owners of identified cases. Data not obtained from the survey was retrieved from medical records and communication with veterinarians directly responsible for case management. Cutaneous SND lesion images were obtained using an iPhone 6 or more recent model with client permission. Ultrasound images were obtained from the Cornell University Hospital for Animals picture archiving and communication service (PACS) by searching for cases with known lesion status. Ultrasound examinations were conducted or supervised by a

board-certified radiologist. Data recorded included: signalment, body weight (initial examination), presence or absence of DM, gross skin lesions (none, mild, fulminant), hepatic ultrasound abnormalities, and histologic reports of skin or liver biopsy specimens. Clinical pathology results were obtained from available medical records. The recorded variables were albumin, ALT, ALP, cholesterol, HCT, and MCV.

2.3 | Statistical analysis

Proportions and percentages were used to describe categorical data. Normality of data was determined using the Anderson-Darling, Agostino & Pearson, Shapiro-Wilk, and Kolmogorov-Smirnov tests. We reported continuous variables with non-Gaussian distributions as median and range and normally distributed data as mean and SD. The chi-squared test compared observed to expected (equal) sex proportions. Principal component (PC) analysis (PCA) was applied to plasma or urine AA profile results. The Kruskal-Wallis test with post hoc Dunn's multiple comparisons test was used to compare clinical pathology variables, plasma 1-methylhistidine, plasma cystathionine, urine lysine, and urine methionine concentrations among groups (eg, control, none, mild, fulminant). Two-way analysis of variance (ANOVA) and Dunn's multiple comparisons were used to compare AA profile results between CON vs ACHES and ACHES-CLF vs ACHES-CLF cases that developed SND. Receiver-operating characteristic (ROC) curve analysis was used to compute sensitivity and specificity of urine biomarkers at which various concentrations and to identify the lowest concentrations that achieved specificity >90%, which were selected as diagnostic thresholds, or thresholds were derived from the univariate ROC results in the Biomarker Analysis tool of Metaboanalyst software 5.0 (https://www.metaboanalyst.ca/MetaboAnalyst/home.xhtml). Commercial software (Prism 9.0 or later, GraphPad, San Diego, California) was used to perform the statistical analyses and to generate corresponding graphs. A P value <.05 established significance.

3 | RESULTS

3.1 | Case demographics

Forty-four dogs with presumed ACHES initially were eligible for inclusion. After excluding 3 cases, 41 dogs were included (Figure 1, Supplementary File 2). No cases had evidence of glucagonoma. Ten cases were treated at our institution, and the remaining cases were managed at various primary care and referral practices. All skin biopsy specimens disclosed the characteristic SND pattern of diffuse parakeratotic hyperkeratosis, subcorneal laminar edema, keratinocyte vacuolation, and basilar hyperplasia as previously described.[10] Similarly, histopathology of the liver disclosed the characteristic pattern of HCS that includes severe glycogen and lipid vacuolation, a moth-eaten appearance of hepatic parenchyma, irregularly marginating proliferative nodules, and distinct areas of parenchymal collapse or parenchymal extinction.[3,10] Plasma AA profile results confirmed the diagnosis in all included cases, and all clinically diagnosed cases additionally had diagnostically compatible urine AA profiles results (Figure 1). Additionally, we encountered no cases with HCH that did not have AA profiles consistent with SND. Various breeds were

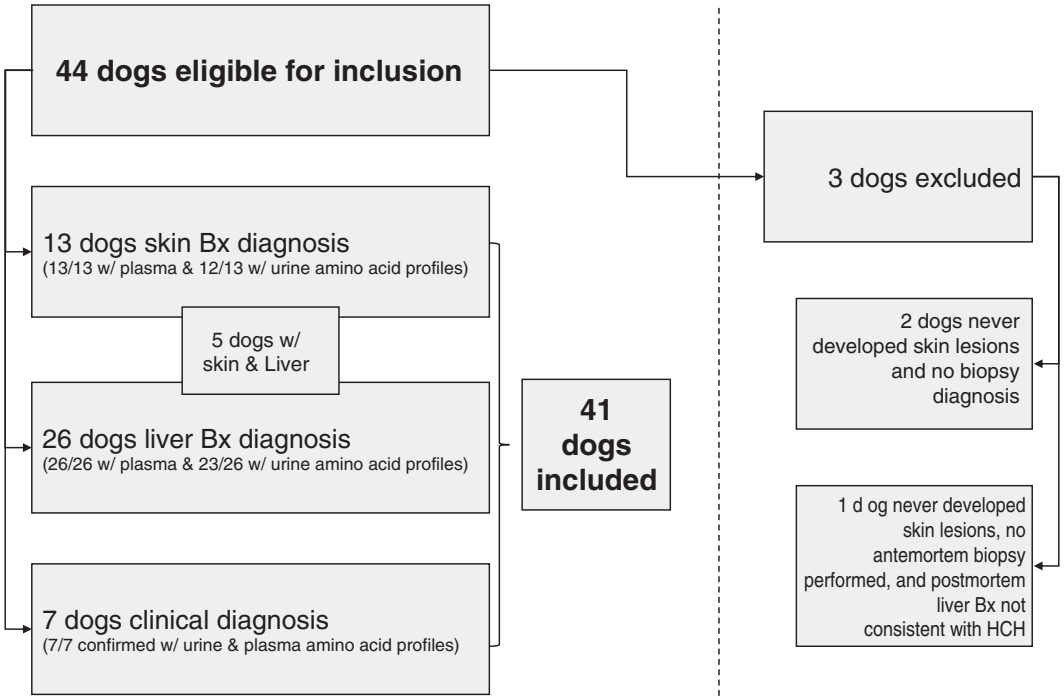


FIGURE 1 Summary of case inclusion, exclusion, and diagnostic methodology. HCH, hepatocutaneous-associated hepatopathy; Bx, biopsy

represented (Supplementary File 2). The most common breeds included West Highland White Terrier (n = 6), Shih Tzu (n = 5), Cocker Spaniel (n = 4), Shetland Sheepdog (n = 4), and 3 each of Chihuahua and Maltese. There were significantly ($P = .04$) more males (neutered, n = 27; intact, n = 1) than females (spayed, n = 14). At diagnosis, the mean age was 9.9 years (SD, 2.2), and the median weight was 10.4 kg (range, 2.3-40.5 kg). The CON group included the following breeds: Cocker Spaniel (n = 2), Shetland Sheepdog (n = 2), Affenpinscher (n = 1), Border Collie mix (n = 1), Cavalier King Charles Spaniel (n = 1), English Pointer (n = 1), Pomeranian (n = 1), Springer Spaniel (n = 1), Standard Poodle (n = 1), and terrier mix (n = 1). Two dogs were diabetic, and their median age was 10 years (range, 8-16 years), and their median weight was 12 kg (range, 3-23 kg).

3.2 | Clinical features

Cutaneous manifestations of SND were variable in ACHES cases, and classified as none, mild (frequently, ambiguous lesions), or fulminant (classic pressure point distribution with erythematous ulcerative oozing or fissured lesions or both). Although 73% (30/41) of dogs developed SND during the observation period, only 54% (22/41) had characteristic, fulminant (Figure 2) skin lesions at diagnosis (Supplementary File 2). After diagnosis (ranging from approximately 1 month to 1 year), 6/13 (69%) ACHES-CLF dogs and 3/6 (50%) dogs with mild cutaneous lesions developed classic SND (Supplementary File 2). Liver ultrasound images were available for all dogs with ACHES diagnosed and managed at our institution (Figure 3). All cases had evident Swiss-cheese-like nodular hepatopathy, regardless of skin lesion status. Five dogs had DM at initial ACHES diagnosis, and another 6 dogs developed DM within 18 months of ACHES diagnosis. Overall, 11/41 (27%) ACHES cases developed DM.

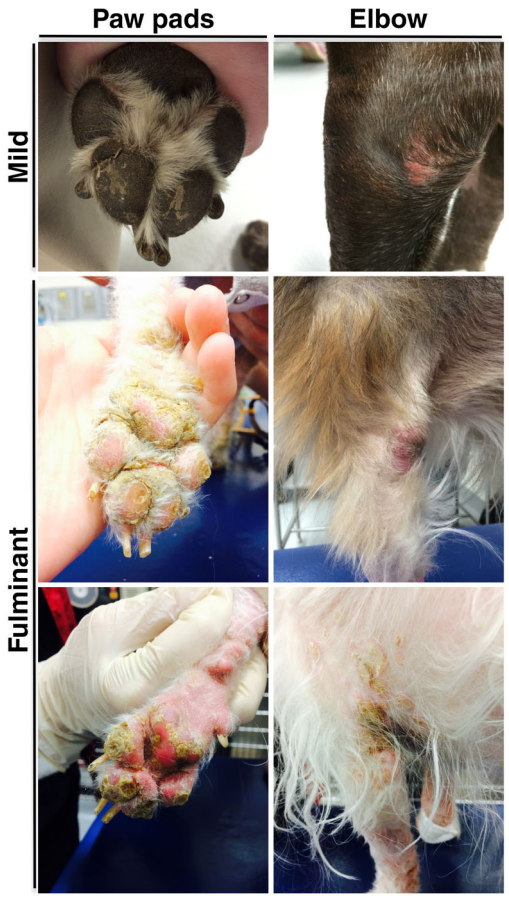


FIGURE 2 Photographic documentation of skin lesions in aminoaciduric canine hypoaminoacidemic hepatopathy syndrome (ACHES) cases. One dog with mild erythema at the point of the elbow bilaterally and mild hyperkeratosis and fissure of the paw pad. Photographs from dogs with fulminant disease exemplify variability in severity of lesions

3.3 | Clinical pathology

Salient clinical pathology variables (HCT, MCV, ALP, ALT, albumin, and cholesterol) obtained at diagnosis were compared among groups categorized by contemporaneous skin lesion status. Data for all selected variables were available for 16 dogs, unavailable for 12 dogs, and partly available for the remaining 13 cases. Dogs with fulminant ACHES had significantly lower HCT ($P = .05$) and MCV ($P = .01$; Figure 4A,B). No differences in ALP, ALT, albumin, or cholesterol were identified (Figure 4C-F) based on skin lesion status at diagnosis.

3.4 | Amino acid profiles

Plasma or urine AA profile results were available for all dogs, and 37 dogs had results for both. Principal component analysis of plasma AA profile results identified clustering of control cases distinctly from 40 ACHES cases (Figure 5A,B) with complete profile data. In contrast, control cases clustered within ACHES cases on PCA of 38 urine AA profile results (Figure 5C,D). Skin lesion status of ACHES cases did not yield distinct clustering regardless of skin lesion status at diagnosis or development of SND at any time (Figure 5).

Plasma concentrations of alanine, glutamine, glycine, lysine, proline, and threonine were significantly lower in ACHES dogs than CON (Supplementary Figure 3A). Plasma AA concentrations were not different between ACHES-CLF dogs and dogs with ACHES-CLF initially that subsequently developed SND (Supplementary Figure 3B). Although not statistically different in combined analysis, 1-methlyhistidine and cystathionine were the most robust plasma biomarkers. Plasma concentrations of 1-methylhistidine were significantly lower in dogs with ACHES than CON when analyzed separately by SND lesion status at diagnosis (Figure 6A; none, $P = .0008$; mild, $P < .0001$; fulminant, $P < .0001$) or anytime (Figure 6B; none, $P = .003$; mild, $P = .0007$; fulminant, $P < .0001$). The same was true for plasma cystathionine analyzed by SND at diagnosis (Figure 6D; none, $P = .009$; fulminant, $P < .0001$) or any time (Figure 6E; none, $P = .003$; mild, $P = .02$; fulminant, $P = .0002$), with the exception of dogs with mild SND lesions at

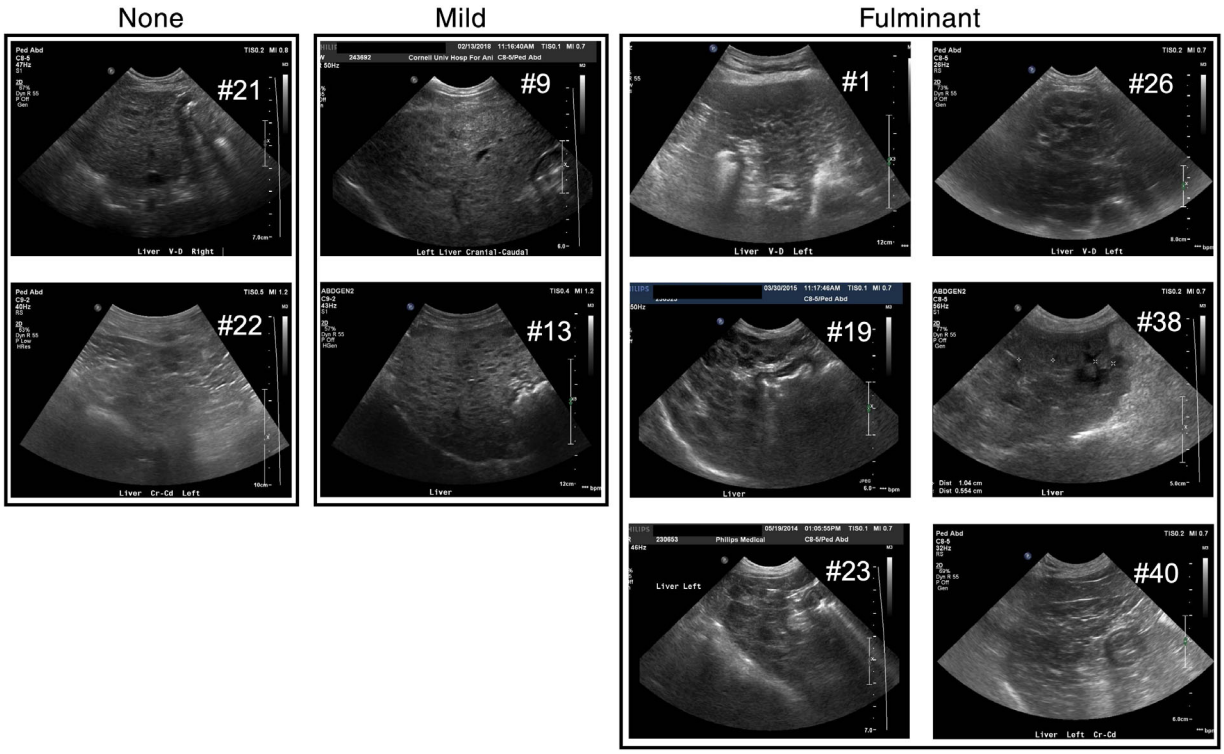
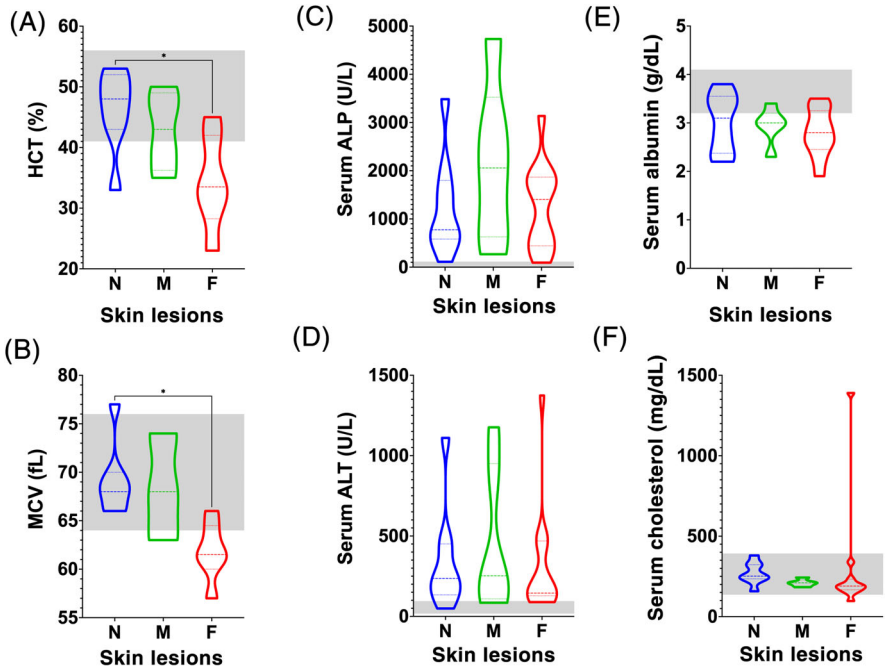


FIGURE 3 Liver ultrasound images of dogs with aminoaciduric canine hypoaminoacidemic hepatopathy syndrome (ACHES). Boxes segregate ultrasound images, representative of each patient's hepatic parenchyma, by skin lesion severity. A board-certified radiologist conducted or supervised all depicted ultrasounds nearest the time of ACHES diagnosis. Numbers indicate patient numbers provided in Supplemental Table 1

FIGURE 4 Salient clinical pathology parameters in dogs with aminoaciduric canine hypoaminoacidemic hepatopathy syndrome (ACHES). Violin plots of hematocrit (HCT, A), mean corpuscular volumes (MCV, B), alkaline phosphatase (ALP, C), alanine aminotransferase (ALT, D), albumin (E), and cholesterol (F) values at diagnosis, segregated by skin lesion severity. N = none, M = mild, F = fulminant. Dashed lines are medians, and dotted lines are quartiles. Shaded regions indicate the reference range from our institution for context. *P < .05



diagnosis, which were not different than CON (P = .08). Diagnostic cut-offs of <7 nmol 1-methylhistidine/mL and < 7.5 nmol cystathionine/mL achieved sensitivities and specificities of 95% and 100%, and 85% and 100%, respectively (Figure 6C,E).

Urine AA PCA loadings plots identified lysine and methionine as large sources of variability in principal component analysis. This finding prompted comparison of urine AAs between CON and ACHES dogs. When all AAs were analyzed, 1-methylhistidine was significantly

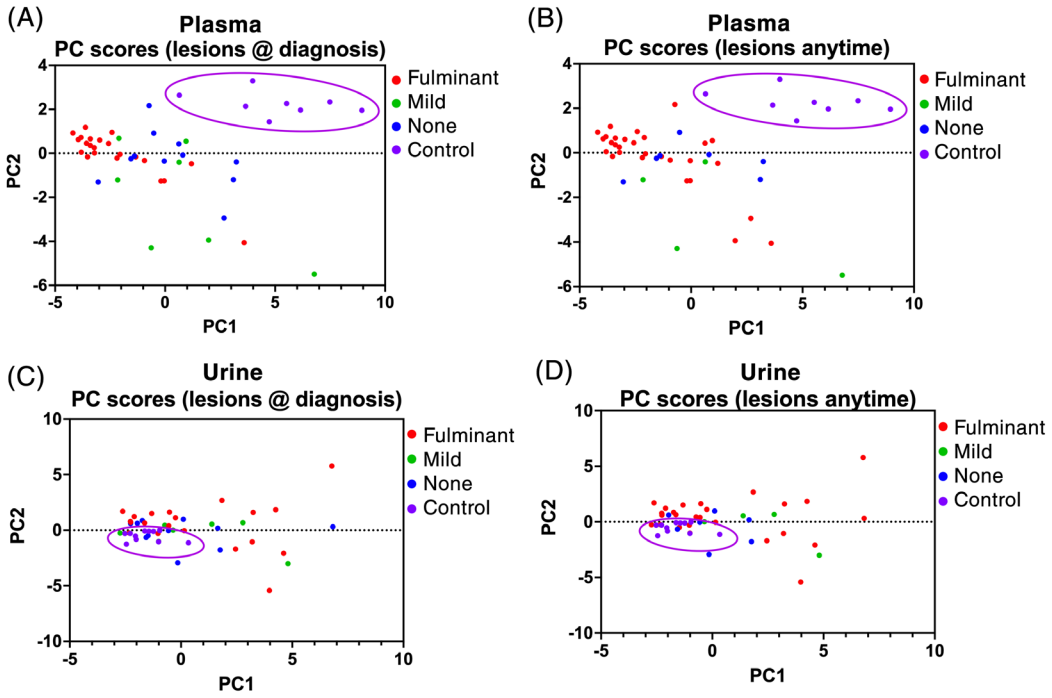


FIGURE 5 Principal component analysis (PCA) of plasma and urine amino acid (AA) profiles in dogs with aminoaciduric canine hypoaminoacidemic hepatopathy syndrome (ACHES). Plots of PCA from plasma (A and B) and urine (C and D) AA profiles. Each dot represents an individual dog with colors identifying control (CON, n = 8 plasma, n = 12 urine) status or skin lesion severity (none, mild, or fulminant) ACHES dogs (n = 40 plasma, n = 38 urine) at the time of diagnosis (A) or reported anytime (B)

different ($P < .0001$) between CON and ACHES (Supplementary File 4A). Absolute concentrations of 1-methylhistidine were substantially higher than any other measured AA. Therefore, a separate analysis excluding measurements of this AA identified a significant difference ($P < .0001$) in urine lysine concentrations between CON and ACHES cases (Supplementary File 4B). Urine lysine, methionine, and 1-methylhistidine concentrations were compared independently among skin lesion groups, and ROC was used to assess their diagnostic biomarker utility. Urine lysine concentrations were significantly higher in ACHES dogs with fulminant lesions at diagnosis ($P = .008$) and in those with mild ($P = .03$) or fulminant ($P = .02$) lesions at any time compared to CON (Figure 7A,B). Urine methionine concentrations were significantly higher in ACHES dogs with fulminant lesions at diagnosis ($P = .0002$) or at any time ($P = .0005$) compared to CON (Figure 7D,E). Results of ROC analysis for 1-methylhistidine refuted its diagnostic utility (data not shown) because of high variability in excreted concentrations in ACHES dogs. Applying thresholds of >344 nmol lysine/mg creatinine and > 68 nmol methionine/mg creatinine yielded sensitivities and specificities of 92% and 63%, and 92% and 74%, respectively (Figure 7C,F).

4 | DISCUSSION

Our purpose was to compare salient features of ACHES-SND and ACHES-CLF cases. We found considerable variability in skin lesion status and severity and SND emerged in a subset of cases initially classified as ACHES-CLF. All ACHES cases reported here were defined by hypoaminoacidemia, and no substantial difference in AA patterns distinguished cases with SND from cases without skin lesions. Anemia and microcytosis were associated with SND lesion status at diagnosis. Subjectively, hepatic ultrasound findings and other objective clinical pathology variables did not differ by presence or absence of SND or SND lesion severity. Similarly, plasma and urine AA profile patterns overlapped between ACHES-SND and ACHES-CLF cases. These findings corroborate our hypothesis that the appearance of SND falls within a continuum of ACHES progression or severity.

The demographics of the cohort included in our study were similar to those previously described in dogs with HCS. Generally, HCS has been more commonly reported in small breed geriatric dogs and had a male predisposition.[3,5,9] Commonly affected breeds include the Cocker spaniel, Shetland sheepdog, Shih Tzu, and West Highland White terrier, as described here.[3,5,9] DM, a recognized comorbidity of HCS, may be present at diagnosis or develop later. Thus, it is essential to assess blood glucose concentrations in these patients frequently. Nearly 30% of dogs in our study were or became diabetic, approximately half developing DM after ACHES diagnosis. The mechanism linking ACHES and DM remains unclear but the first description of the syndrome established DM as a comorbidity.[2] Although most cases of SND in dogs are not associated with glucagonoma, when measured, glucagon concentrations in affected dogs are within reference limits or mildly increased.[10,14-16] In the context of hypoaminoacidemia,

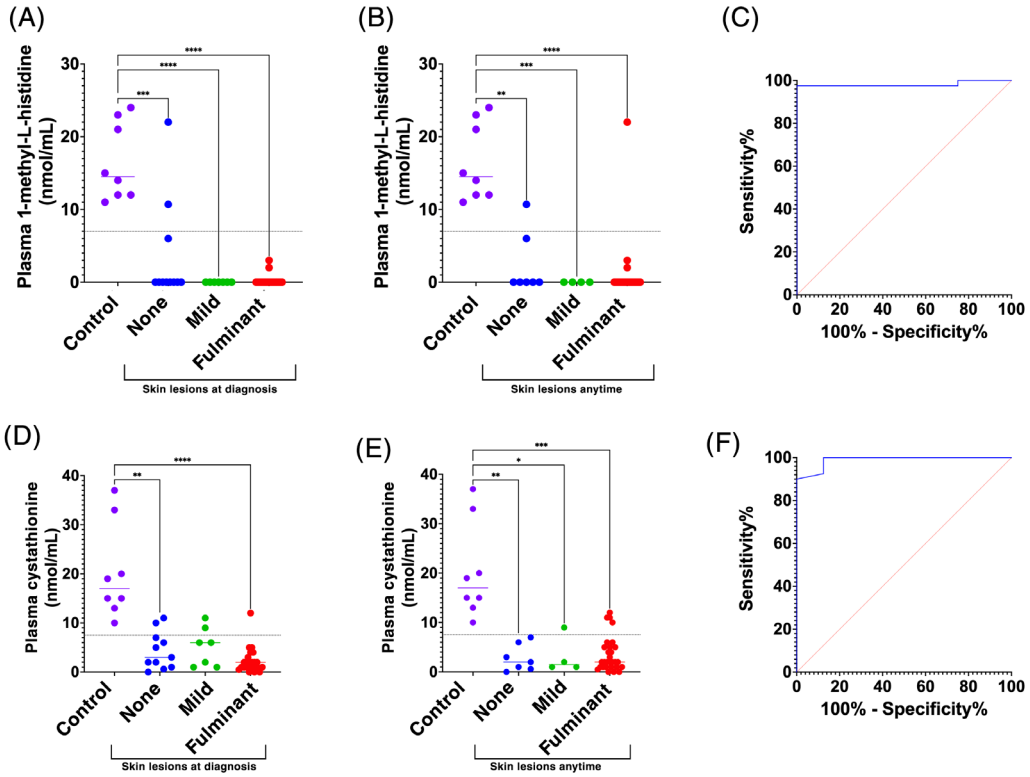
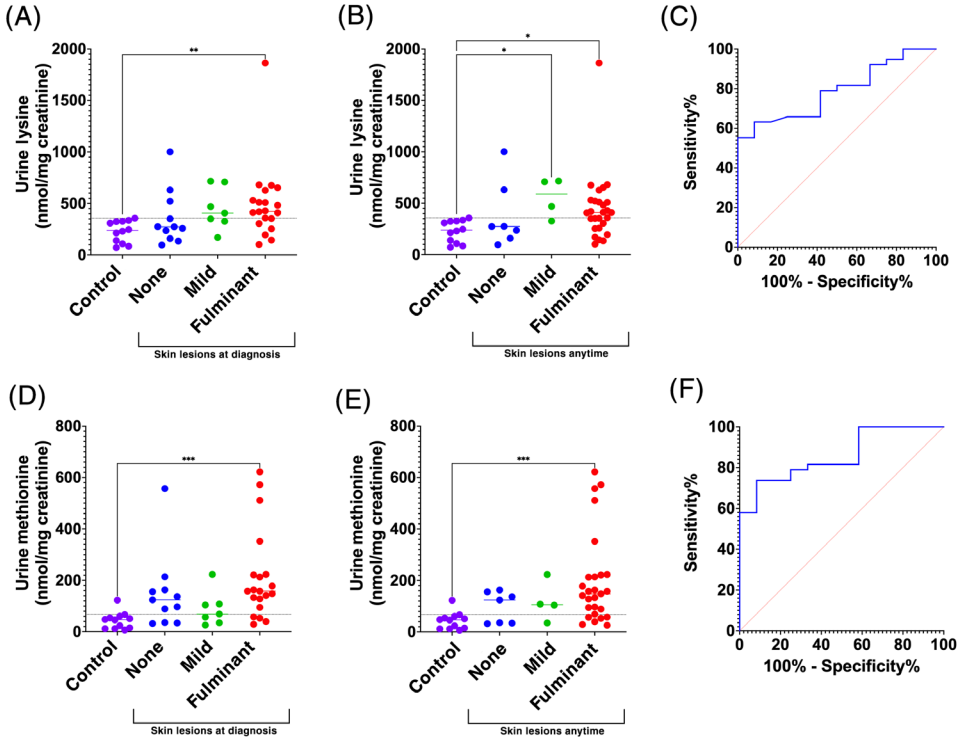


FIGURE 6 Plasma 1-methylhistidine and cystathionine concentrations in aminoaciduric canine hypoaminoacidemic hepatopathy syndrome (ACHES). Plasma 1-methylhistidine (A and B) and cystathionine (D and E) concentrations in ACHES cases segregated by skin lesion severity at the time of diagnosis (A and D) or observed at any time (B and E). Dotted horizontal lines (A, B, D, and E) indicate diagnostic biomarker cutoffs for plasma 1-methylhistidine and cystathionine (<7 or <7.5 nmol/mL, respectively). *P < .05, **P < .01, ***P < .001, ****P < .0001. Receiver-operating characteristics of plasma 1-methylhistidine (C) and cystathionine (F)

FIGURE 7 Urine lysine and methionine concentrations in aminoaciduric canine hypoaminoacidemic hepatopathy syndrome (ACHES). Creatinine-normalized urine lysine (A and B) and methionine (D and E) concentrations in ACHES cases segregated by skin lesion severity at the time of diagnosis (A and D) or observed at any time (B and E). Dotted horizontal lines (A, B, D, and E) indicate diagnostic biomarker cutoffs for urine lysine and methionine (> 344 or >68 nmol/mg creatinine, respectively). *P < .05, **P < .01, ***P < .001. Receiver-operating characteristics of urine lysine (C) and methionine (F)



normal blood glucagon concentrations are likely inappropriate.[17] Hyperglucagonemia drives hypoaminoacidemia, and glucagon-secreting tumors can cause SND. Thus, relative hyperglucagonemia (eg, normal plasma concentrations in a dog with hypoaminoacidemia) may explain the central syndromic features of ACHES and the predisposition to DM.

Our case series emphasizes why clinicians should consider ACHES in dogs with HCH lesions, but lacking typical SND. Although characteristic SND skin lesions dominated the clinical description of HCS for over 3 decades,[2,4,9,10] 46% of dogs in our study lacked apparent cutaneous lesions (ie, none or mild SND lesions) at initial diagnosis, supporting our recommended terminology of ACHES. In some dogs initially diagnosed with HCH by biopsy alone, classic SND skin lesions subsequently may emerge (20% of cases in our study) at variable intervals.[5] We propose considering HCH as a differential diagnosis for dogs with severe degenerative nodular vacuolar hepatopathy, detected either by ultrasound imaging or hepatic biopsy. Several distinctive histologic features define HCH, as previously described and illustrated in Shih Tzus with this syndrome.[3] Although a grading scheme theoretically would be beneficial for studying this syndrome, the severe nature of the vacuolar hepatopathy and presence of other features does not readily allow hepatic histologic grading of HCH.

Abnormal clinical pathology findings reported previously for dogs with SND or HCS include anemia, microcytosis, increased ALP activity, and hypoalbuminemia.[5] Increased ALP activity was observed in ACHES cases regardless of cutaneous lesion status. Anemia and microcytosis were most severe in dogs with fulminant ACHES, suggesting a relationship between SND and these hematologic changes. Microcytic anemia occurs in people with protein-calorie malnutrition and in approximately one-third of individuals with kwashiorkor (protein-calorie malnutrition).[18] Indeed, microcytic anemia is 1 of the only laboratory abnormalities present in children with kwashiorkor secondary to fad diets in the United States.[19] Thus, it is reasonable to speculate that persistent hypoaminoacidemia drives a form of protein-calorie malnutrition in ACHES that can manifest as SND or microcytic anemia, frequently occurring together.

Plasma and urine AA profiling provides a noninvasive method for disease confirmation.[5,9,13] As previously demonstrated, creatinine-normalized urine AA profiling, documenting lysinuria, provides a novel diagnostic metric that may augment the diagnostic utility of plasma AA profiling.[5] Our data indicate further that plasma AA profiles are similar among ACHES cases, regardless of skin lesion status, facilitating its diagnosis in cases without SND. Despite the significant difference in urine 1-methyl-histidine concentrations between ACHES and CON dogs, the wide range of concentrations measured in ACHES cases precluded this urine AA as a diagnostically valuable urine biomarker. We identified pathologic methioninuria as a robust diagnostic marker for ACHES. Reference range results for urine methionine concentrations were higher than those of the CON cohort in our study, obscuring abnormal urine concentrations of this AA in a previous study.[5,20] Urine lysine concentrations in the CON dogs were higher than previously reported in apparently healthy dogs more than 40 years ago[20] that provided reference data that established lysinuria as a feature of HCS.[5] This realization increases the diagnostic threshold approximately 3-fold for ACHES relative to what was reported previously.[5] Although an explanation could be that the CON dogs included cases of subclinical ACHES, all of the CON dogs with plasma AA profiles had unremarkable results and no other evidence of ACHES. We are unaware of a specific reason for changes in reference urine AA concentrations, but possibilities could range from updated dog food formulations to evolving AA assay methods during the following 40 years. Periodic assessment of urine AA concentrations from appropriate reference cases may be needed to adjust diagnostic cutoffs. Similarly, new plasma AA biomarkers (1-methylhistidine and cystathionine) were identified in our study using biomarker identification software and ROC analysis that was facilitated by a control cohort. This information adds to a previous study that investigated hypoaminoacidemia and aminoaciduria in dogs with HCS utilizing reference results alone.[5]

The causal mechanism for hypoaminoacidemia in ACHES remains unclear, but the prevalence of DM as a comorbidity combined with aminoaciduria may offer clues. The proglucagon-derived hormones glucagon, glucagon-like peptide-1, and glucagon-like peptide-2 are essential regulators of glucose and AA homeostasis. Hyperglucagonemia drives hypoaminoacidemia, and GLP-2 regulates AA transporter function.[17,21-23] It is interesting to consider if dysregulation in this hormone axis could contribute to the pathomechanisms of ACHES. Dysregulation of AA transport and hypoaminoacidemia may be linked to SND lesions by depletion of substrates essential for maintaining normal integument or by other previously proposed mechanisms.[9,10] Prolonged hypoaminoacidemia could be required before a threshold is reached for skin lesion development.

Retrospective data collection is an important limitation of our study. Some records were incomplete or lost between initial case identification and completion of our study. Some assessments, such as skin lesion severity, were subjective and were made by different clinicians. This subjectivity must be considered a possible source of bias in our study, but this bias most likely was applied to categorizing mild vs fulminant skin lesions. Future studies including an expanded control group or groups that include diabetic dogs and dogs with severe degenerative glycogen-type vacuolar hepatopathies resembling HCS are needed. Unfortunately, these considerations were beyond the logistical and financial scope of our study. However, previous reports measuring plasma AA concentrations in dogs and people with various liver diseases suggest that the pattern and severity of hypoaminoacidemia is unique to ACHES.[9,24,25]

Our study provides a rationale for applying a new acronym (ACHES) to replace HCS, because some dogs lack cutaneous lesions at the time of syndrome diagnosis. The proposed ACHES acronym reflects the clinical spectrum of disease manifestations and mandates HCH, hypoaminoacidemia, and aminoaciduria as defining characteristics. Other syndrome manifestations such as DM and SND can emerge at any time after the initial ACHES diagnosis, if not evident initially. Diagnosing ACHES early, before SND onset may improve survival as a result of earlier intervention, as suggested another study.[26] Our findings indicate that clinicians should consider plasma and urine AA profiles in dogs with severe degenerative vacuolar hepatopathies

associated with Swiss cheese-like nodular ultrasonographic patterns in the liver and persistent markedly increased ALP activity, even in the absence of SND. Further investigation of this syndrome hopefully will improve approaches to its diagnosis and treatment.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the Cornell University IACUC, protocol 2017-0094.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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