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4 **Prednisolone therapy for chronic hepatitis in English Springer Spaniels: A prospective study of**
5 **12 cases.**

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Abstract:

Background: English Springer Spaniels (ESS) show an increased risk of chronic hepatitis (CH). In a previous study of 68 ESS with CH, in which only one dog received corticosteroids, a median survival time of 189 days was noted. Some ESS with CH appear to improve with prednisolone treatment, therefore we aimed to investigate the response to prednisolone in this breed.

Participants: ESS with histologically confirmed idiopathic CH were treated with prednisolone 1-2mg/kg/day. Nine female and three male ESS were enrolled (median age at diagnosis of five years). Patients were monitored clinically and had biochemistry samples taken to assess markers of hepatocellular damage and function.

Results: The mean starting dose of prednisolone was 1.1mg/kg/day. All symptomatic patients showed an initial clinical improvement. Two cases were euthanased while receiving prednisolone. The median time since diagnosis is 1,715 days [range: 672-2,105 days] and the remaining patients are clinically well, with seven patients still receiving a mean dose of 0.4mg/kg prednisolone every other day. Statistical analysis demonstrated significant ($P<0.05$) reductions in serum ALKP, ALT and bilirubin following 2-4 weeks of prednisolone treatment.

Conclusion: This study demonstrates improved clinical and biochemical parameters when some ESS with CH are managed with prednisolone and standard supportive treatments.

Keywords: Hepatology; Canine; Corticosteroids

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59 **Introduction:**

60 English Springer Spaniels (ESS) in the United Kingdom have an increased risk of chronic hepatitis
61 (CH)¹. CH is defined by the World Small Animal Veterinary Association (WSAVA) Liver
62 Standardisation Project² as histological evidence of hepatocellular apoptosis, necrosis, regeneration,
63 predominant mononuclear cell infiltration and fibrosis. The reported post-mortem prevalence of CH in
64 dogs in first-opinion practice is 12%, suggesting this is a common disease which likely has a range of
65 aetiologies including nutritional, environmental, genetic and infectious³. There are several well-
66 documented causes of canine CH, such as copper accumulation due to a defect in copper metabolism⁴
67 and possible infectious causes due to *Bartonella spp*⁵, *Leptospira spp*⁶ and *Helicobacter spp*⁷.
68 Although previous studies have identified viral causes of CH, including canine adenovirus type I⁸
69 there is currently no substantial evidence to suggest viral causes are a significant aetiology for canine
70 CH⁹⁻¹². Studies have been performed which support an immune-mediated aetiology to CH in some
71 breeds¹³⁻¹⁴. CH has a number of well-reported breed predispositions including the Labrador retriever¹⁵,
72 American cocker spaniel¹⁶, English cocker spaniel¹⁷, ESS¹, Dalmatian¹⁸, Doberman pinscher¹⁹, Great
73 Dane²⁰, Cairn terrier and Samoyed¹, however the underlying aetiology is frequently unknown and
74 therefore treatment often remains non-specific and supportive²¹.

75 In a previous study of 68 ESS with biopsy confirmed idiopathic CH, only one was treated with
76 prednisolone, and the median survival time of the whole cohort was 189 days (range: 1 – 1,211
77 days)²². This suggested that the underlying disease process in the ESS was aggressive and rapidly fatal
78 in most cases, in contrast to a previous study looking at 79 dogs of various breeds with histologically
79 confirmed CH which reported mean survival times of 21.1 to 36.4 months when cirrhosis was not
80 present²³. Research investigating the disease in ESS and other breeds in the UK initially concentrated
81 on attempts to find a viral cause for the disease because of the histological similarity to human viral
82 hepatitis and canine acidophil cell hepatitis^{9, 24}. Corticosteroid treatment was not initially advised and

83 cases were instead managed supportively. However, the progression of CH in these cases remained
84 mostly rapid and short survival times were noted²², yet some clinicians reported improved survival
85 when these cases were given corticosteroids. Despite the widespread use of corticosteroids in the
86 general treatment of canine CH, only two previous studies have investigated their efficacy²⁵⁻²⁶. One of
87 those studies was published prior to the WSAVA Liver Standardisation Project, which generates
88 concern that some patients were not truly idiopathic, and both studies included a range of canine
89 breeds. Although both studies identified some clinical and biochemical improvements in some cases
90 of canine CH, it is likely that the study populations included a diverse range of underlying disease
91 processes and therefore the results are likely difficult to interpret. There are no currently published
92 studies investigating the response to prednisolone in canine patients with CH in a single breed.

93 Therefore, the authors instituted a prospective cohort study aimed at investigating the clinical and
94 biochemical response to prednisolone and other supportive treatments in a group of ESS with
95 histopathologically confirmed idiopathic CH.

96

97 **Materials and Methods:**

98 ESS being treated in first-opinion practice, or referred to the Queen's Veterinary School Hospital
99 (University of Cambridge) with a histological diagnosis of idiopathic CH were enrolled prospectively
100 between 2009-2017. No cases had previously been involved in studies investigating CH in ESS. Cases
101 were identified when veterinary surgeons contacted the authors for advice. A histopathological
102 diagnosis was based on a predominantly lymphoplasmacytic, interface hepatitis and variable fibrosis,
103 and according to WSAVA criteria for a diagnosis of CH. All liver biopsies were stained with
104 rhodanine for qualitative copper assessment using a previously published copper grading system²⁷.
105 Samples were scored as grade 1: absence or few copper-containing granules in the cytoplasm of an
106 occasional hepatocyte; grade 2: obvious copper-containing granules in some centrilobular
107 hepatocytes; grade 3: numerous granules in most centrilobular hepatocytes (one-third of each lobule);
108 grade 4: presence of numerous granules in all centrilobular and midzonal hepatocytes (approximately

109 two-thirds of the hepatocytes in all lobules); grade 5: abundant granules in more than two-thirds of the
110 liver cells in all lobules. The histopathology specimens were examined by several board-certified
111 pathologists and were excluded if significant copper accumulation (grade 3-5) was documented. Cases
112 with evidence of pyogranulomatous hepatitis were also excluded. No cases had been treated with
113 corticosteroids within six months of the study. Attending veterinary surgeons gave prednisolone 1-
114 2mg/kg/day and submitted haematology and biochemistry samples and progress reports to the authors.
115 The prednisolone starting dose range was based on two previous studies that investigated the response
116 to prednisolone in various canine breeds with chronic hepatitis²⁵⁻²⁶. Prednisolone is a well-reported
117 treatment option for canine CH, and the decision to start the patients on this therapy was at the
118 discretion of the clinician in charge of each case. Informed owner consent was obtained for analysis of
119 patient data. All patients had their first recheck 2-4 weeks following initiation of prednisolone
120 therapy, which included biochemical assessment of liver parameters. Further blood samples were
121 advised to be taken prior to any prednisolone dose reduction. The prednisolone dose was tapered by
122 25-50% every 4 weeks according to the patient's clinical and biochemical response, focusing on
123 alkaline phosphatase (ALKP), alanine aminotransferase (ALT) and bilirubin. While all cases had
124 these values assessed at diagnosis and at the first recheck, not all cases had these values measured
125 each time the prednisolone dose was altered. To account for variation in instruments used to assess
126 biochemical parameters, and corresponding variation in reference ranges, the ALKP, ALT and
127 bilirubin have been presented as multiples of the upper limit of the reference range for the individual
128 machine used. Due to individual patient variation in the way prednisolone was tapered and timing of
129 blood samples, the biochemical values were plotted against the prednisolone dose at the time of
130 sampling rather than against specific time points. Thus the values were recorded and plotted
131 graphically against different oral doses of prednisolone, including the values at diagnosis of CH; the
132 values after prednisolone 1-2mg/kg/day for 2-4 weeks; 0.5-1mg/kg/day; 0.5-1mg/kg/EOD; 0.25-
133 0.5mg/kg/EOD; after cessation of prednisolone treatment (if applicable); and after re-starting
134 prednisolone treatment if cessation of medication caused a relapse in clinical signs. Additional
135 therapies used included combinations of s-adenosylmethionine, silybin, ursodeoxycholic acid,
136 antibiotics and hepatic diet.

137 *Statistical analysis:*

138 Shapiro-Wilk normality testing was performed on the data and identified a lack of normal distribution,
139 typical of small data sets. Therefore, the non-parametric two-sided Wilcoxon test was used to
140 demonstrate significant ($P < 0.05$) changes in serum ALKP, ALT and bilirubin following prednisolone
141 therapy.

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143 **Results:**

144 Sixteen cases of suspected ESS CH were evaluated during the study period. Two cases were excluded
145 because hepatic biopsies were not performed, and two cases were excluded because their
146 histopathology results were not consistent with the previously published WSAVA criteria for CH.

147 Nine female and three male ESS were enrolled with a median age at diagnosis of five years [range: 11
148 months-10 years]. All cases had been diagnosed following evaluation of wedge liver biopsies taken
149 during laparotomy or laparoscopy. Within these cases one dog was being treated with levothyroxine
150 for hypothyroidism at the time of recruitment, and one case was subsequently diagnosed with protein-
151 losing nephropathy eight months after initially presenting for hepatic disease, and her urine protein
152 creatinine ratio improved following treatment with standard doses of benazepril (0.5mg/kg SID PO).

153 Four of the 12 cases showed no clinical signs of CH at diagnosis but were investigated after routine
154 blood sampling for an unrelated reason detected elevations of liver enzymes. Seven of the 12 cases
155 had bile culture performed and no bacteria were cultured, however the remaining five patients did not
156 have this evaluated. Table 1 summarises the histopathological diagnosis for each of the 12 ESS cases
157 included in the study. None of the 12 ESS displayed significant qualitative copper accumulation
158 (Table 1) and therefore quantitative copper analysis was not performed in these cases. Furthermore,
159 there was no histopathological evidence of significant biliary tract inflammation in any of the
160 evaluated samples from the 12 cases. The mean prednisolone starting dosage was 1.1mg/kg/day
161 [range: 1.0-2.0mg/kg/day]. Symptomatic patients showed a subjective improvement clinically within
162 four weeks according to the owners. Table 2 reports the clinical abnormalities reported for each case,

163 both at enrolment and at the first recheck appointment, as well as additional treatments provided to
164 each patient at the time of diagnosis. Two out of the 12 cases were euthanased due to CH-related signs
165 while receiving prednisolone, with survival times of 122 and 741 days from diagnosis. Clinical signs
166 that prompted euthanasia for these two patients included hepatic encephalopathy, melaena, jaundice
167 and lethargy. The remaining ten patients are alive and clinically well at the time of manuscript
168 submission, with seven patients still receiving a mean dosage of 0.4mg/kg prednisolone every other
169 day (EOD) [range: 0.25mg/kg/EOD – 1mg/kg/day]. Three patients stopped prednisolone therapy
170 without a concurrent elevation in liver parameters, while three cases stopped and needed to be
171 restarted on prednisolone due to recurrence of CH-related signs or an elevation in liver parameters. At
172 the time of submission, four patients are currently in the process of having their prednisolone dose
173 reduced with the aim to stop and monitor for recurrence of clinical signs. The median time since
174 diagnosis for the ten remaining cases is 1,715 days [range: 672-2,105 days].

175 Table 3 presents the median values for ALKP, ALT and bilirubin at diagnosis of CH, as well as the
176 values at the patients' first recheck 2-4 weeks after starting prednisolone. Two-sided Wilcoxon test
177 demonstrated a significant reduction in ALKP, ALT and bilirubin at the first recheck following
178 prednisolone treatment with p-values 0.0010, 0.002 and 0.0156 respectively. Due to variability in the
179 length of time patients remained on the tapering doses of prednisolone, the authors elected not to
180 assess for significant changes between the remaining prednisolone doses.

181 Figures 1 and 2 depict the serum values for ALKP and ALT respectively, from the 12 ESS cases in
182 this study. In all dogs there were elevations in ALKP and ALT prior to prednisolone treatment, but
183 following initiation of prednisolone therapy all values significantly reduced for all patients. However,
184 in the 11 patients that had oral prednisolone reduced to 0.25-0.5mg/kg/EOD or stopped entirely, seven
185 (64%) documented an increase in either or both ALKP and ALT. In the three cases that had ALKP
186 measurements following re-starting prednisolone after cessation of treatment, the ALKP values were
187 subjectively decreased (Figure 1), and the same was found for measured ALT (Figure 2). In five of
188 the twelve ESS cases, the measured serum ALKP never returned to within the reference range.

189 Furthermore, we found that nine of the twelve cases documented serum ALT that did not return to
190 within the reference range, despite resolution of clinical signs.

191 Figure 3 presents the values of serum bilirubin in the nine ESS that had these values measured. The
192 initial values are elevated prior to prednisolone treatment in six patients, and there is a significant
193 reduction in these values following initiation of prednisolone. Five of the six patients with elevated
194 serum bilirubin showed a return to normal range, and the one patient whose elevated bilirubin did not
195 return to normal is still early in the treatment course and has shown a substantial decrease which is
196 approaching the reference interval.

197

198 **Discussion:**

199 This study documents that some ESS with histologically confirmed idiopathic CH show clinical and
200 clinicopathological improvement to prednisolone 1-2mg/kg/day, in addition to standard supportive
201 treatments. The median time since diagnosis in our current study was 1,715 days (range: 672 - 2,105
202 days) and although we cannot make direct comparisons, it does appear that the ESS in our study had
203 an improved survival compared with the previously documented median survival time of 189 days
204 (range: 1 - 1,211 days)²². Unfortunately, we do not have a direct control population for comparison,
205 however given the aggressive nature of ESS CH, and the previously published benefits of
206 prednisolone for canine CH^{25 - 26}, we felt it was inappropriate to deny patients medication that could
207 benefit them. As a result, we must acknowledge that the supportive treatments provided to the patients
208 may have contributed to our results. Furthermore, our results suggest that serial measurements of
209 ALKP, ALT and serum bilirubin are useful for monitoring the patient's response to prednisolone
210 therapy, and the increase in some dogs when prednisolone therapy was stopped further supports their
211 use. This is the first study providing evidence for the use of prednisolone in some ESS with CH and
212 indeed the first study documenting corticosteroid response in a single rather than multiple breeds²⁶.
213 This positive response was convincing in spite of the absence of a control population without
214 treatment. These were different dogs from those described in the previous study²² and offers

215 additional support for a female predisposition with nine of the 12 cases being female. Interestingly,
216 two previous studies^{1,22} identified a young to middle-aged onset of disease in ESS (median age at
217 diagnosis of five years and 3 years 7 months, respectively) which is similar to the median age at
218 diagnosis in our current study of five years. This is younger than the overall median age of 8 years in
219 a study of 551 dogs of varying breeds with CH in the UK¹ which had a total of 551 dogs of varying
220 breeds with CH in the UK. Histologically the liver tissue from ESS CH cases show a predominant
221 lymphoplasmacytic inflammation with interface hepatitis, variable fibrosis that can extend between
222 portal triads and hepatocellular apoptosis and necrosis. Whilst it would have been interesting to assess
223 liver histopathology following treatment with prednisolone, this was not evaluated in the current
224 study. Table 1 summarises the histopathological diagnosis for each of our 12 ESS cases and the
225 features identified are remarkably similar to those expected with human autoimmune hepatitis
226 (AIH)²⁸. Hepatic histopathology alone is not considered diagnostic for AIH in humans, but instead
227 further validation is required with response to immunosuppressive drugs and positive detection of
228 various serum autoantibodies including non-organ-specific and organ-specific autoantibodies such as
229 anti-nuclear (ANA), smooth muscle (SMA), liver cytosol type-I (LC-1) and liver-kidney-microsomal
230 type-I (LKM-1) antibodies²⁹. Human leucocyte antigen (HLA) alleles which confer an increased risk
231 for developing AIH have been found in affected individuals³⁰. These alleles have also been shown to
232 influence progression of the disease, which is interesting in light of the previously documented
233 association between dog leukocyte antigen (DLA) and CH in the ESS³¹. Regarding assessment of
234 hepatic copper, none of the 12 cases were reported to have significant copper accumulation following
235 qualitative copper grading, however it is possible that variation between the pathologists resulted in a
236 degree of interobserver variation. However, all pathologists were board-certified and as such are very
237 likely to have made the authors aware if they had concerns regarding the qualitative copper
238 assessment of the histopathology specimens.

239 An unexpected finding in this study was that four of the 12 cases were perceived to be
240 asymptomatic at the time of diagnosis, in contrast to a previous case series suggesting that the disease
241 is usually aggressive and rapidly fatal²². These patients may have been identified early in the course of

242 their disease and it is possible they will have become clinically unwell in the future if the disease had
243 not been investigated. Human AIH has an asymptomatic presentation in 25-34% cases³², with 26-70%
244 of these patients going on to develop clinical signs within 32 months of diagnosis. The number of
245 asymptomatic cases in our current study is not too dissimilar from that reported in human literature
246 which could suggest some similarities between human AIH and ESS CH. It is impossible to know in
247 either humans or dogs how long a patient may be asymptomatic prior to clinical presentation because
248 patients without symptoms are not routinely blood tested. An equally unexpected finding was the
249 significant reduction in ALKP in patients despite prednisolone therapy. It is known that
250 corticosteroids induce ALKP activity in dogs, and therefore it is common for patients treated with
251 prednisolone to experience increased serum concentrations of the enzyme³³. The significant reduction
252 in serum ALKP seen in our cohort following initiation of prednisolone treatment suggests that, in
253 these cases, the initial enzyme elevation prior to corticosteroid administration was primarily disease
254 induced. Therefore, controlling the disease with prednisolone appeared to result in a corresponding,
255 significant reduction in hepatocellular damage and cholestasis. The continued mild elevations in ALP
256 on treatment are consistent with steroid induction of enzymes supported by the fact that bilirubin
257 became normal in six cases.

258 We recognise there are limitations to this investigation. Due to the clinical nature of this cohort study,
259 with individual patients being managed by different veterinarians, there was some variability in the
260 way prednisolone was tapered and timing of blood samples. Therefore, the authors did not plot the
261 measured serum values of ALT, ALKP and bilirubin against time from initiation of treatment, but
262 instead the values were plotted against different doses of prednisolone. Furthermore, all cases
263 received additional supportive medications for CH which varied between patients and could have
264 influenced results. However, this variability is inherently difficult to overcome when dealing with
265 patients in clinical practice and also made it challenging to accurately standardise a clinical scoring
266 system. It is also important to note that cases were enrolled at different times which resulted in
267 patients being at different stages of their disease with some having fully recovered while others
268 having more recently been diagnosed and started on medication at the time of manuscript submission.

269

270 **Conclusion:**

271 This study documents that some ESS with histologically confirmed idiopathic CH show clinical and
272 clinicopathological improvement to prednisolone 1-2mg/kg/day, in addition to standard supportive
273 dietary and medical management. Further studies are indicated to investigate potential serum markers
274 of autoimmunity and the use of other immunosuppressive treatments in affected dogs.

275

276 **Conflict of Interest statement:**

277 None of the authors have any financial or personal relationships that could inappropriately influence
278 or bias the content of this paper.

279

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286 **References:**

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373 **Tables:**

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375 **Table 1:** Histopathological diagnosis for each of the twelve English Springer Spaniels included in the
376 study.

Patient:	Histopathological diagnosis:
1	Hepatitis, interface, lymphocytic, plasmacytic and neutrophilic, chronic, mild, with moderate to marked hepatocyte apoptosis. Grade 1 copper.
2	Hepatitis, periportal, lymphocytic and neutrophilic, chronic, marked, with moderate porto-portal bridging fibrosis. Grade 2 copper.
3	Hepatitis, lymphocytic, plasmacytic and neutrophilic, chronic, moderate, with moderate hepatocyte apoptosis and necrosis. Grade 2 copper.
4	Hepatitis, interface, lymphocytic, plasmacytic and neutrophilic, chronic, severe, with mild porto-portal bridging fibrosis. Grade 2 copper.
5	Hepatitis, periportal, lymphocytic and neutrophilic, chronic, marked with moderate hepatocyte apoptosis and necrosis. Grade 2 copper.
6	Hepatitis, periportal, lymphocytic, plasmacytic and neutrophilic, chronic, marked, with mild porto-portal bridging fibrosis. Grade 1 copper.
7	Hepatitis, interface, lymphocytic, plasmacytic and neutrophilic, chronic, severe, with mild biliary hyperplasia and hepatocyte necrosis. Grade 2 copper.
8	Hepatitis, lymphocytic, plasmacytic, chronic, moderate, with moderate porto-portal bridging fibrosis. Grade 1 copper.
9	Hepatitis, lymphocytic, plasmacytic, chronic, moderate, with moderate portal fibrosis and portal biliary hyperplasia. Grade 1 copper.
10	Hepatitis, lymphocytic, plasmacytic, subacute, moderate. Grade 2 copper.
11	Hepatitis, lymphocytic, plasmacytic and neutrophilic, subacute, moderate, with occasional pigmented histiocytes and mild hepatocyte apoptosis. Grade 2 copper.
12	Hepatitis, lobular and interface, lymphocytic and neutrophilic, chronic, severe with mild portal fibrosis. Grade 1 copper.

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389 **Table 2:** Clinical signs documented for each of the twelve English Springer Spaniels reported in the
 390 study, both at enrolment and at the first recheck appointment (2-4 weeks after initiation of
 391 prednisolone therapy) with additional treatments:

Patient:	Clinical signs at diagnosis:	Clinical signs at first recheck:	Additional treatments:
1	Reduce appetite, vomiting, jaundice.	Resolution of jaundice and no abnormal clinical signs currently reported by owner.	UDCA, SAmE, amoxicillin-clavulanate
2	PUPD.	Resolution of PUPD according to owner.	UDCA, SAmE
3	Anorexia, PUPD, vomiting, weight-loss, jaundice, lethargy.	Jaundice still identified during clinical examination and appetite improved but reduced compared to normal. Resolution of vomiting, PUPD and lethargy according to owner, however weight-loss continued. The patient subsequently developed neurological abnormalities with worsening jaundice, and was euthanised 122 days after initiating prednisolone therapy.	UDCA, SAmE, metronidazole
4	Lethargy, reduced appetite, vomiting, jaundice.	Jaundice not identified during clinical examination and no abnormal signs currently reported by the owner.	UDCA, SAmE
5	No abnormal clinical signs reported by owner.	Still reported to be clinically normal by owner.	UDCA, SAmE, hepatic diet
6	No abnormal clinical signs reported by owner.	Still reported to be clinically normal by owner.	SAmE, hepatic diet
7	No abnormal clinical signs reported by owner.	Still reported to be clinically normal by owner.	SAmE
8	Jaundice, ascites, weight-loss.	Resolution of jaundice and ascites during clinical examination. No abnormal signs currently reported by the owner. The patient subsequently developed neurological abnormalities and melaena, and was euthanised 741 days after initiating prednisolone therapy.	UDCA, SAmE, spironolactone
9	Reduced appetite and PUPD.	PUPD still present but improved, according to	UDCA, SAmE

		owner. Appetite now normal.	
10	No abnormal clinical signs reported by owner.	Still reported to be clinically normal by owner.	
11	Vomiting, lethargy, jaundice, ascites, PUPD.	Mild PUPD and polyphagia reported by owner, otherwise no abnormal clinical signs.	Hepatic diet
12	Lethargy, diarrhoea, vomiting.	Resolution of vomiting and diarrhoea but owner still reported mild lethargy.	SAMe, hepatic diet, cefalexin

392 *PUPD: polyuria/polydipsia; UDCA: ursodeoxycholic acid; SAMe: S-adenosylmethionine

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416 **Table 3:** Median values for serum alkaline phosphatase (ALKP), alanine aminotransferase (ALT) and
417 bilirubin in English Springer Spaniels at diagnosis of CH, and at first recheck (2-4 weeks after
418 initiation of prednisolone 1-2mg/kg/day).

	Median value* at diagnosis (range)	Median value* at first recheck (range)	P-Value
ALKP	8.5 (3.7 - 16.2)	2.7 (1.1 - 8.3)	0.0010
ALT	10.8 (2.5 – 46.3)	3.9 (0.8 – 14.7)	0.0020
Bilirubin	1.4 (0.3 – 27.9)	0.45 (0.1 – 4.1)	0.0156

425 **The values are reported as a multiple of the upper limit of the reference range.*

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449 **Figure legends:**

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451 **Figure 1:** Serum [ALKP] as a multiple of the upper limit of the reference range in 12 ESS with CH, at
452 diagnosis (before prednisolone treatment), first recheck (2-4 weeks after starting 1-2mg/kg/day
453 prednisolone) and at tapering doses of prednisolone. A significant difference was identified between
454 the values at diagnosis and first recheck, however due to variability of dosing the authors did not
455 assess statistical differences between the remaining prednisolone doses. Each coloured shape
456 represents an individual patient. *EOD: every other day.

457

458 **Figure 2:** Serum [ALT] as a multiple of the upper limit of the reference range in 12 ESS with CH, at
459 diagnosis (before prednisolone treatment), first recheck (2-4 weeks after starting 1-2mg/kg/day
460 prednisolone) and at tapering doses of prednisolone. A significant difference was identified between
461 the values at diagnosis and first recheck, however due to variability of dosing the authors did not
462 assess statistical differences between the remaining prednisolone doses. Each coloured shape
463 represents an individual patient. *EOD: every other day.

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465 **Figure 3:** Figure 3: Serum [bilirubin] as a multiple of the upper limit of the reference range in 9 ESS
466 with CH, at diagnosis (before prednisolone treatment), first recheck (2-4 weeks after starting 1-
467 2mg/kg/day prednisolone) and at tapering doses of prednisolone. A significant difference was
468 identified between the values at diagnosis and first recheck, however due to variability of dosing the
469 authors did not assess statistical differences between the remaining prednisolone doses. Each coloured
470 shape represents an individual patient. *EOD: every other day.