Serum thyroxine and thyroid-stimulating hormone concentration in hyperthyroid cats that develop azotaemia after radioiodine therapy

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OBJECTIVES: The objectives of this study were to determine which serum thyroid hormone test best identifies iatrogenic hypothyroidism in cats that develop azotaemia after radiiodine treatment and to determine which thyroid test best differentiates these azotaemic, hypothyroid cats from azotaemic, radiiodine-treated euthyroid cats, as well as from azotaemic cats with chronic kidney disease and no history of thyroid disease.

MATERIALS AND METHODS: A total of 42 hyperthyroid cats that developed azotaemia (serum creatinine ≥220 µmol/L) after radiiodine treatment had serum concentrations of thyroxine and free thyroxine by dialysis and thyroid-stimulating hormone measured at 3, 6 and 12 months. Iatrogenic hypothyroidism was confirmed (n=28) or excluded (n=14) on the basis of thyroid scintigraphy. A total of 14 cats with chronic kidney disease and 166 clinically normal cats underwent similar serum thyroid testing and scintigraphy.

RESULTS: Concentrations of thyroxine and free thyroxine were lower and thyroid-stimulating hormone higher in hypothyroid cats than in all three groups of euthyroid cats (P<0.0001). Of the hypothyroid cats, thyroxine and free thyroxine concentrations were low in 15 (53-6%) and seven (25%), respectively. Low serum thyroxine and free thyroxine concentrations were also detected in seven (50%) and two (14-3%) of the cats with chronic kidney disease. Thyroid-stimulating hormone concentrations were elevated in all hypothyroid cats but remained within the reference interval in all three groups of euthyroid cats. Serum thyroid-stimulating hormone had a higher test sensitivity and specificity than either thyroxine or free thyroxine concentration.

CLINICAL SIGNIFICANCE: The finding of high serum thyroid-stimulating hormone concentrations best identifies feline iatrogenic hypothyroidism and differentiates it from non-thyroidal illness syndrome in cats that develop azotaemia after treatment.
INTRODUCTION

Radioiodine ($^{131}$I) is considered the treatment of choice for feline hyperthyroidism but can cause iatrogenic hypothyroidism in 3 to 79% of cats (Meric & Rubin 1990, Boag et al. 2007) with most studies reporting a prevalence of approximately 10% (Klausner 1987, Craig 1993, Peterson & Becker 1995, Chun et al. 2002, Nykamp et al. 2005). However, cats with iatrogenic hypothyroidism only rarely develop overt clinical signs (e.g., lethargy, hair-coat changes) consistent with hypothyroidism (Peterson & Becker 1995, Peterson 2016), and there are no research guidelines to help clinicians best determine how to diagnose or manage iatrogenic hypothyroidism in these cats.

Treating hyperthyroidism can also unmask subclinical chronic kidney disease (CKD), with 17 to 49% of cats developing azotaemia within three to six months of treatment (Graves et al. 1994, Adams et al. 1997b, Becker et al. 2000, Slater et al. 2001, Riensche et al. 2008, Milner et al. 2006, Boag et al. 2007, Williams et al. 2010a, Vaske et al. 2016). These cats have underlying, non-azotaemic CKD when hyperthyroid but become azotaemic after successful treatment of the hyperthyroidism. Cats with iatrogenic hypothyroidism are more likely to develop azotaemic CKD than treated cats that remain euthyroid (Williams et al. 2010a). More importantly, iatrogenic hypothyroidism might reduce survival time, especially in cats with concurrent azotaemia (Williams et al. 2010a). Therefore, despite the fact that most hypothyroid cats display few, if any, clinical signs, thyroid hormone replacement might preserve kidney function and improve survival in these cats.

Studies have generally diagnosed feline iatrogenic hypothyroidism when serum total thyroxine ($T_4$) concentrations fall below the laboratory reference interval following treatment (Graves et al. 1994, Nykamp et al. 2005, Boag et al. 2007). However, diagnosis of hypothyroidism based exclusively on baseline $T_4$ concentrations is problematic because non-thyroidal illnesses (including CKD) can lower serum $T_4$ concentrations (Peterson & Gamble 1990, Mooney et al. 1996, Peterson et al. 2001, Davignon et al. 2015). Therefore, cats that develop subnormal or borderline serum $T_4$ concentrations together with newly developed azotaemia after $^{131}$I treatment represent a diagnostic challenge. Because iatrogenic hypothyroidism can contribute to the decline in renal function (Iglesias & Díez 2009, Van Hoek & Daminet 2009, Mariani & Berns 2012), diagnostic tests that distinguish iatrogenic hypothyroidism from low $T_4$ concentrations associated with non-thyroidal illness are needed.

Measuring free $T_4$ (fT4) concentration (together with total $T_4$ concentration) is currently recommended for diagnosing hypothyroidism in cats with concurrent non-thyroidal disease (Peterson 2013a). However, non-thyroidal illness in cats can also lower fT4 concentrations (Peterson et al. 2001), limiting the diagnostic utility of this test. This is compounded by the use of various methods for measuring fT4 (e.g., equilibrium dialysis, radioimmunoassay, chemiluminescence) by diagnostic laboratories (Schachter et al. 2004, Peterson 2013b, Randolph et al. 2015), with consequent variable diagnostic test performance, especially in cats with non-thyroidal illness (Mooney et al. 1996, Peterson et al. 2001, Peterson 2013b, Davignon et al. 2015). Because of these problems with interpretation and measurement of $T_4$ and fT4 concentrations, several investigators have used a canine thyroid-stimulating hormone (cTSH) assay to measure serum TSH concentrations in cats with suspected hypothyroidism – mostly cats with congenital hypothyroidism (Blois et al. 2010, Williams et al. 2010a, Lim et al. 2014, Galgano et al. 2014, Peterson 2015). The diagnosis of spontaneous hypothyroidism in these cats was generally based on finding low serum total $T_4$ and fT4 and high TSH concentrations. However, measurement of feline serum TSH concentration with the canine assay is not universally accepted, and there are no studies reporting the diagnostic accuracy of serum TSH concentrations in cats with iatrogenic hypothyroidism.

Many human patients in whom thyroid dysfunction is being investigated have normal serum concentrations of total $T_4$ and fT4 but high serum TSH concentrations (Canaris et al. 2000, Hollowell et al. 2002, Aoki et al. 2007, Empson et al. 2007, Hennessey & Espallart 2015). These patients have what is most commonly referred to as subclinical hypothyroidism, which, by definition, is a biochemical diagnosis because few, if any, clinical features of thyroid failure are present (Cooper 2001, Vanderpump & Tunbridge 2002, Fatourechi 2009, Garg & Vanderpump 2013). Subclinical hypothyroidism is also a common finding following $^{131}$I therapy in human patients with Graves’ disease and toxic nodular goiter (Vanderpump & Tunbridge 2002, Biondi & Cooper 2008, Garg & Vanderpump 2013). Similarly, a high prevalence of iatrogenic subclinical hypothyroidism was recently reported in cats treated with $^{131}$I (Lucy et al. 2017) based on the findings of normal serum $T_4$ but high TSH concentrations.

In this study, we sought to determine which serum thyroid hormone test best identifies iatrogenic hypothyroidism (both overt and subclinical) in cats that initially develop azotaemia after $^{131}$I treatment. Furthermore, we sought to determine which test would best differentiate these azotaemic, hypothyroid cats from azotaemic, euthyroid cats following $^{131}$I treatment, as well as from azotaemic cats with CKD but no history of thyroid disease, by comparing test sensitivities and specificities of serum $T_4$, fT4 and TSH concentrations for diagnosis of iatrogenic hypothyroidism in cats. Finally, we examined the effects of levothyroxine replacement therapy on serum $T_4$, fT4, TSH and creatinine concentrations in azotaemic cats with iatrogenic hypothyroidism.

MATERIALS AND METHODS

Study design and selection of animals

We enrolled four groups of cats for this prospective study: (A) hyperthyroid cats treated with $^{131}$I that subsequently developed moderate azotaemia (serum creatinine ≥220µmol/L) and iatrogenic hypothyroidism, (B) hyperthyroid cats treated with $^{131}$I that subsequently developed moderate azotaemia (serum creatinine ≥220µmol/L) but remained euthyroid, (C) azotaemic cats with spontaneous CKD and (D) clinically normal cats. Ethical
approval for the study was granted by our Institutional Animal Ethics Committee, and all procedures were performed after obtaining informed owner consent.

**Hyperthyroid cats treated with $^{131}$I that subsequently developed azotaemia (n=42)**

All hyperthyroid cats referred for treatment with $^{131}$I from June, 2013 to June, 2015 were evaluated for inclusion in this part of the study. To be eligible for inclusion, untreated hyperthyroid cats underwent a thorough evaluation that included review of the past medical record, complete physical examination, routine laboratory testing [complete blood count (CBC) and serum biochemical profile], determination of serum thyroid hormones (total $T_4$, $fT_4$, and TSH) (Peterson et al. 2015) and qualitative and quantitative thyroid scintigraphy (Peterson 2015, Peterson & Broome 2015, Peterson et al. 2016b). We excluded cats with pre-existent azotaemia (defined as serum creatinine ≥175 µmol/L), either when hyperthyroid or controlled with methimazole, or if other concurrent non-thyroidal disease was present. A total of 524 $^{131}$I-treated cats were considered eligible (pre-treatment serum creatinine <175 µmol/L) and were enrolled in this study (Fig 1).

![Flowchart](Flowchart.png)
Following 131I treatment, 42 cats that either remained hyperthyroid or did not return for follow-up monitoring were excluded (Fig 1). The remaining 478 cats were re-evaluated at 3, 6 and 12 months by measurement of serum concentrations of creatinine, T4, fT4, and TSH. Of these, 50 developed moderate azotaemia (defined as serum creatinine ≥220 µmol/L, consistent with International Renal Interest Society stages 2b to 3 CKD) that was persistent and generally progressive, verified on two or more occasions at one- to three-month intervals (Elliott & Watson 2014). In these azotaemic cats, repeat quantitative thyroid scintigraphy was used to determine thyroid status (i.e. euthyroidism versus hypothyroidism), with a scintigraphic diagnosis of hypothyroidism based on qualitative assessment of diminished or absent accumulation of radionuclide by the thyroid gland, with “less-than-normal” amounts of residual thyroid tissue (Peterson 2013a), as well as quantitative analysis and finding of low values for at least three of the following variables: thyroid-to-salivary gland ratio, thyroid-to-tracheal background ratio, percent thyroidal uptake of the administered 99mTc-pertechnetate and calculated thyroid volume (Table 1). Of the 42 cats re-evaluated with thyroid scintigraphy, 28 were judged hypothyroid, and 14 were euthyroid; 8 of the 50 azotaemic cats did not undergo follow-up quantitative scintigraphy and were excluded (Fig 1).

Of the 28 cats with documented hypothyroidism, 19 were available for follow-up after being treated with levothyroxine. Serum concentrations of creatinine, T4, fT4, and TSH were measured after at least one month of levothyroxine treatment to determine the influence of replacement therapy on these analytes.

Cats with naturally occurring CKD (n=14)

For inclusion in this study, these cats had to have moderate azotaemia (defined as serum creatinine ≥220 µmol/L; Table 1), but there was no clinical evidence of thyroid disease (i.e. none had palpable thyroid nodules, and all had thyroid scintigraphy documenting normal thyroid function) or history of hyperthyroidism. All of these cats had blood collected once for determination of serum T4, fT4, and TSH concentrations (Table 1).

### Clinically normal, euthyroid cats (n=166)

These cats were recruited as controls as well as to establish reference intervals for serum T4, fT4, TSH and creatinine concentrations. They had to be at least seven years of age and considered healthy based on an unremarkable client history, physical examination (i.e. none had palpable thyroid nodules), CBC, serum chemistry profile, urinalysis and thyroid scintigraphy (Table 1).

### Assays for thyroid hormone, thyrotropin (TSH) and creatinine concentrations

Blood samples were collected from all cats to determine serum concentrations of total T4, fT4, by dialysis, TSH and creatinine. Serum creatinine was measured using a modified Jaffe method (Jacobs et al. 1991) on an automated biochemistry analyser (AU5400 Clinical Chemistry System, Beckman Coulter), whereas T4, fT4 and TSH were determined by assays validated for use in cats as previously described (Peterson et al. 2015). For the TSH assay, the lower limit of quantification was 0·03 ng/mL, and the upper limit of the reportable values is 12 ng/mL; the reference interval for feline TSH concentration ranged from less than 0·03 to 0·3 ng/mL (Peterson et al. 2015).

All blood samples were centrifuged within one hour after collection; serum was separated and stored at 4°C until assayed by a commercial laboratory (Antech Diagnostics) the following day.

### Data and statistical analyses

Data were assessed for normality by the D’Agostino-Pearson test and by visual inspection of graphical plots. Data were not normally distributed; therefore, all analyses used non-parametric tests. Results are reported as median [interquartile range (IQR), 25th to 75th percentile] and are represented graphically as box- and-whisker plots. For all analyses, statistical significance was defined as P≤0·05.

All statistical analyses were performed using proprietary statistical software (GraphPad Prism, version 6.0; GraphPad Software). Data from the control cats were used to establish reference intervals for serum concentrations of T4, fT4, TSH and creatinine.

### Table 1. Median (interquartile range) values for the T/S ratio, T/TB ratio, TcTU, thyroid volume and serum creatinine concentrations in 28 azotaemic cats with 131I-induced hypothyroidism, 14 euthyroid-azotaemic cats previously treated with 131I and 14 euthyroid cats with CKD

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cats with CKD (14)</th>
<th>Cats treated with 131I for hyperthyroidism</th>
<th>Reference interval (RI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T/S ratio</td>
<td>0·70 (0·59 to 1·04)</td>
<td>0·64 (0·53 to 1·40)</td>
<td>0·35 to 2·75</td>
</tr>
<tr>
<td>Number (%) below RI</td>
<td>0 (0%)</td>
<td>25 (89·3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>T/TB ratio</td>
<td>1·97 (1·75 to 2·24)</td>
<td>1·83 (1·68 to 1·94)</td>
<td>1·50 to 4·0</td>
</tr>
<tr>
<td>Number (%) below RI</td>
<td>0 (0%)</td>
<td>28 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>TcTU (%)</td>
<td>0·40 (0·32 to 0·60)</td>
<td>0·37 (0·21 to 0·63)</td>
<td>0·15 to 0·85</td>
</tr>
<tr>
<td>Number (%) below RI</td>
<td>0 (0%)</td>
<td>25 (89·3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Volume (gm)</td>
<td>0·75 (0·66 to 0·81)</td>
<td>0·66 (0·60 to 0·80)</td>
<td>0·30 to 1·0</td>
</tr>
<tr>
<td>Number (%) below RI</td>
<td>0 (0%)</td>
<td>21 (75%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Serum creatinine (µmol/L)</td>
<td>270 (237 to 303)</td>
<td>256 (246 to 276)</td>
<td>80 to 170</td>
</tr>
</tbody>
</table>

CKD Chronic kidney disease, T/S Background-adjusted thyroid-to-salivary ratio, T/TB Thyroid-to-tracheal background ratio, TcTU Percent thyroidal uptake of 99mTc-pertechnetate T/S ratio was calculated by dividing the average thyroid count density (minus background count density) by the salivary count density (minus background count density); T/TB ratio was calculated by dividing thyroid count density by tracheal-background count density; the TcTU calculated by dividing the net thyroid counts of all visible thyroid tissue by the number of radioactive counts injected. Thyroid volume was estimated using the equation for a spheroid, as previously described.

*Value statistically different (P<0·0001) from both azotaemic, euthyroid cats and cats with kidney disease
creatinine by a non-parametric method to identify the central 95th percentile interval (i.e. 2.5 through 97.5th percentile range) (Friedrichs et al. 2012). Continuous variables (e.g. serum thyroid hormone concentrations) were compared between groups by the Kruskal–Wallis test, followed by Dunn’s multiple comparisons test. Comparisons between two measurements within groups (before–after) were analysed with the Wilcoxon signed rank test. Diagnostic test sensitivity and specificity were calculated for each hormone (T₄, fT₄, and cTSH); McNemar’s test (Trajman & Luiz 2008) for paired or dependent proportions was then used to determine whether differences existed between the sensitivity or specificity of serum T₄, fT₄, and cTSH as diagnostic tests for iatrogenic hypothyroidism in cats. For the hypothyroid cats, median survival times for the group of cats treated with levothyroxine versus the group not treated were determined by Kaplan–Meier product-limit method (Machin et al. 2006). Survival curves were plotted for cats in each group and compared by the log-rank (Mantel–Cox) test with right censoring. Median follow-up times for the two groups of hypothyroid cats were also calculated and compared using the Mann–Whitney test.

RESULTS

Cat groups

Azotaemic cats with ¹³¹I-induced hypothyroidism (n=28)

The hypothyroid cats ranged in age from 8 to 17 years (median, 14.0 years). Breeds included domestic longhair and shorthair (n=26), Siamese and Ragdoll (one cat each). Of these, 16 were female, and 11 were male; all had been neutered. Before treatment for hyperthyroidism, the median serum T₄ concentration in these cats was 103 nmol/L (reference interval, 12 to 50 nmol/L); thyroid scintigraphy confirmed bilateral thyroid disease in 15 cats and unilateral disease in 13. The median ¹³¹I dose administered was 74 mBq. At re-evaluation (median time, 6-5 months; IQR, 5 to 9 months; range, 3 to 13 months), 26 of the 28 cats had gained weight (median body weight increased from 4.6 to 5.2 kg). Owners of 10 (71.4%) of the cats reported increased polyuria, polydipsia or both. No clinical signs of hypothyroidism were noted. The two groups of ¹³¹I-treated cats did not differ in age, pre-treatment serum T₄ concentration, prevalence of unilateral and bilateral disease or dose of ¹³¹I administered.

Of the 28 azotaemic cats that developed iatrogenic hypothyroidism, 19 were subsequently rechecked after being treated with 150 µg/day per os levothyroxine, with post-pill serum concentrations of T₄, fT₄, TSH and creatinine monitored after a median (IQR) of 2.0 (1.3 to 5.6) months. Of the remaining nine hypothyroid cats, five owners declined levothyroxine treatment, two owners agreed to begin levothyroxine but failed to return their cat for follow-up, and two owners were unable to administer the oral levothyroxine to their cats.

Euthyroid cats with CKD (n=14)

The cats with CKD ranged in age from 7 to 19 years (median, 14.0 years). Breeds included domestic longhair and shorthair (nine cats), Himalayan (two cats) and Maine Coon, Persian and Siamese (one cat each). Seven cats were female, and seven were male.

Clinically normal, euthyroid cats (n=166)

These cats ranged in age from 7 to 18 years (median, 9.0 years). Breeds included domestic longhair and shorthair (152 cats), American shorthair (3 cats), Siamese (2 cats), Abyssinian, Bengal, Burmese, Chartreux, Maine Coon, Ragdoll, Russian blue, Scottish fold and Tonkinese (1 cat each). Of these cats, 86 (51.8%) were female, and 80 were male; all had been neutered.

Serum thyroid hormone and TSH concentrations

Serum T₄ concentrations

Serum T₄ concentrations in azotaemic cats with iatrogenic hypothyroidism (median, 11.6 nmol/L) did not differ from cats with CKD (median, 11.0 nmol/L) (P=0.98); both groups had lower serum T₄ concentrations than euthyroid ¹³¹I-treated cats (median, 23.2 nmol/L) and healthy cats (median, 27.0 nmol/L) (P<0.0001; Fig 2). The euthyroid ¹³¹I-treated cats had lower serum T₄ concentrations than clinically normal cats (P=0.035).

A total of 15 (53.6%) hypothyroid cats had low serum T₄ concentrations, and 13 (46.4%) had T₄ concentrations within the reference interval (Fig 1). These 13 cats all had T₄ concentrations in the lower half of the reference interval (i.e. between 13 and 19 nmol/L) (Fig 1). In contrast, seven (50%) euthyroid cats with CKD and three (1.9%) healthy cats had low serum T₄ concentrations (Fig 2). None of the euthyroid ¹³¹I-treated cats had low serum T₄ concentrations.

Serum fT₄ concentrations

Serum fT₄ concentrations did not differ between azotaemic cats with iatrogenic hypothyroidism (median, 15 nmol/L) and cats...
with CKD (median, 15 nmol/L) (P=0.72); both groups had serum \( fT_4 \) concentrations lower than euthyroid \( ^{131} \)I-treated cats (median, 25.5 nmol/L) and healthy cats (median, 31 nmol/L) (P<0.0001; Fig 3). Finally, euthyroid \( ^{131} \)I-treated cats had lower serum \( fT_4 \) concentrations than healthy cats (P=0.013).

Seven (25%) hypothyroid cats had low serum \( fT_4 \) concentrations, and 21 (75%) had \( fT_4 \) concentrations within the reference interval (Fig 3). These 21 cats all had \( fT_4 \) concentrations in the lower half of the reference interval (i.e. between 11 and 24 pmol/L) (Fig 1). In contrast, three (21.4%) euthyroid cats with CKD and one (0.6%) healthy cat had low serum \( fT_4 \) concentrations (Fig 3). None of the euthyroid \( ^{131} \)I-treated cats had low \( fT_4 \) concentrations.

**Serum TSH concentrations**

Azotaemic cats with iatrogenic hypothyroidism had higher TSH concentrations (median, 3.3 ng/mL) than euthyroid \( ^{131} \)I-treated cats (median, 0.11 ng/mL), cats with CKD (median, 0.05 ng/mL) or healthy cats (median, 0.04 ng/mL) (P<0.0001 for all comparisons) (Fig 4). The euthyroid \( ^{131} \)I-treated cats had higher serum TSH concentrations than either healthy cats (P<0.0001) or cats with CKD (P=0.02).

All 28 hypothyroid cats had high serum TSH concentrations. In contrast, serum TSH concentrations remained within the reference interval in all euthyroid cats with CKD and euthyroid \( ^{131} \)I-treated cats. Only three (1.9%) healthy cats had slightly high serum TSH concentrations (Fig 4).

**Diagnostic test sensitivity and specificity of \( T_4 \), \( fT_4 \) and TSH concentrations**

A high serum TSH concentration showed higher sensitivity for identifying iatrogenic hypothyroidism than either low serum \( T_4 \) or low serum \( fT_4 \) concentrations (P<0.001) (Table 2). A low
serum $T_4$ concentration showed higher sensitivity for identifying iatrogenic hypothyroidism than low serum $fT_4$ concentration ($P=0.039$). Similarly, a normal serum TSH concentration showed higher specificity for excluding iatrogenic hypothyroidism than either the normal $T_4$ or $fT_4$ concentrations ($P<0.0001$) (Table 2).

**Before and after L-T$_4$ supplementation to cats with iatrogenic hypothyroidism**

After levothyroxine supplementation, serum $T_4$ and $fT_4$ concentrations increased in all 19 iatrogenic hypothyroid cats (Fig 5A, B). Concurrently, serum TSH concentrations decreased in all

### Table 2. Calculation of diagnostic test sensitivity and specificity for serum concentrations of $T_4$, $fT_4$ and TSH in 28 azotaemic cats with $^{131}I$-induced hypothyroidism; 14 azotaemic, euthyroid cats previously treated with $^{131}I$; 14 cats with CKD and no history of thyroid disease; and 166 clinically normal cats

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypothyroid (28)</td>
<td>Euthyroid, $^{131}I$-treated (14)</td>
</tr>
<tr>
<td>Serum $T_4$</td>
<td>53·6% (33·9 to 72·5)</td>
<td>100% (76·8 to 100)</td>
</tr>
<tr>
<td>Serum $fT_4$</td>
<td>25·0% (10·7 to 44·9)</td>
<td>100% (76·8 to 100)</td>
</tr>
<tr>
<td>Serum TSH</td>
<td>100% (87·7 to 100)</td>
<td>100% (76·8 to 100)</td>
</tr>
</tbody>
</table>

CI Confidence intervals, CKD Chronic kidney disease, $T_4$ Thyroxine, $fT_4$ Free thyroxine, TSH Thyroid-stimulating hormone

**Fig 5.** Line charts of serum concentrations of (A) $T_4$ (thyroxine), (B) free thyroxine ($fT_4$), (C) thyroid-stimulating hormone (TSH) and (D) creatinine in 19 hypothyroid cats before and after treatment with levothyroxine (L-$T_4$)
roxine was also longer than in the untreated cats (Fig 6). The median survival time in the cats treated with levothyroxine was 346 to 798 days, which was significantly longer (P=0.001; Fig 6) than the median survival time in the 9 untreated cats (median, 220 days; IQR, 102 to 369 days). Of the nine untreated hypothyroid cats, two remained alive at 182 and 237 days with worsening azotaemia. Overall, the time from date of hypothyroid diagnosis to last follow-up in the 19 levothyroxine-treated cats (median, 550 days; IQR, 346 to 798 days) was significantly longer (P=0.001; Fig 6) than the time in the 9 untreated cats (median, 220 days; IQR, 102 to 369 days). The median survival time in the cats treated with levothyroxine was also longer than in the untreated cats (Fig 6).

**DISCUSSION**

Our results indicate that measurement of serum TSH concentration is a very sensitive and specific diagnostic test for iatrogenic (131I-induced) hypothyroidism in cats that develop azotaemic CKD after treatment. Compared to both serum T4 and fT4 concentrations, serum TSH concentration is better at both diagnosing hypothyroidism and in differentiating hypothyroid cats from euthyroid cats with CKD. After levothyroxine supplementation of cats with hypothyroidism, high serum TSH concentrations decreased to within the reference interval, indicating that serum TSH measurement can also aid in monitoring levothyroxine therapy (Maeda et al. 1976).

Hyperthyroidism increases glomerular filtration rate (GFR) (Graves et al. 1994, Boag et al. 2007, van Hoek et al. 2009, Vaske et al. 2016) and decreases body muscle mass (Peterson et al. 2016a), both of which lower serum creatinine concentrations. In some cats, this can “mask” concurrent azotaemic CKD, which only becomes evident once high-circulating thyroid hormone concentrations (and GFR) fall to normal or low levels. For inclusion in this study, we selected a population of hyperthyroid cats with masked CKD, i.e. all had serum creatinine concentrations within the reference interval at the time of hyperthyroid diagnosis but developed azotaemia (≥220 µmol/L) only after 131I treatment. We initially chose this cut-off value for serum creatinine because it is the upper limit of the reference interval reported by the diagnostic laboratory used in this study. However, we re-established the reference interval using data from our clinically normal cats and lowered the upper limit to 175 µmol/L. Our goal was to examine 131I-treated cats that developed unequivocal and repeatable elevations in creatinine concentrations because it is critical in this cohort of cats to distinguish iatrogenic hypothyroidism (which can be treated with levothyroxine) from the low serum thyroid hormone concentrations that can develop in cats with CKD and other non-thyroidal illnesses (Peterson & Gamble 1990, Peterson et al. 2001, van Hoek et al. 2010, Davignon et al. 2015). We saw considerable overlap in both serum T4 and fT4 concentrations in azotaemic, hypothyroid cats and euthyroid cats with CKD, but serum TSH concentration clearly differentiated these populations.

The proportion of cats that develop azotaemia after treatment for hyperthyroidism depends, at least in part, on the degree to which the hyperthyroidism is controlled (i.e. how low the serum T4 or fT4 fall) (Williams et al. 2010b). Importantly, azotaemia is more likely to develop in cats with overt iatrogenic hypothyroidism (defined as a low total T4 with high TSH concentration) than in cats with subclinical hypothyroidism (low to normal total T4 with high TSH concentration) (Lucy et al. 2017) or in 131I-treated cats that become euthyroid (Williams et al. 2014, Lucy et al. 2017). As with those studies, our 42 131I-treated cats with unmasked, moderate azotaemic CKD were twice as likely to be hypothyroid than to be euthyroid – essentially, two-thirds of all cats with “unmasked” azotaemic CKD were hypothyroid, and only a third were euthyroid.

The azotaemia that develops in cats with iatrogenic hypothyroidism also appears to negatively impact survival. In one study, overtly hypothyroid, azotaemic cats had shorter (456 days) median survival than hypothyroid, non-azotaemic cats (905 days) (Williams et al. 2010a). Additionally, based on the results of this study, hypothyroid (overt or subclinical) cats not given thyroid hormone replacement had a shorter median survival than did the hypothyroid cats supplemented with levothyroxine, suggesting a beneficial effect of thyroid hormone replacement on survival. Thus, we believe it is important to identify and correct iatrogenic hypothyroidism if it develops, especially in cats with unmasked, moderate azotaemic CKD.

In one study of azotaemic cats that developed iatrogenic hypothyroidism following methimazole overdosage, lowering the daily dose to restore euthyroidism also led to a fall in creatinine.
concentrations, with azotaemia resolving in half of the cats (Williams et al. 2014). Similarly, levothyroxine replacement in our cats with iatrogenic hypothyroidism (both overt and subclinical) led to significant decreases in serum creatinine concentrations. Although the mechanism(s) for how levothyroxine treatment improves renal function in cats is not completely known, the decrease in creatinine concentrations is likely related to thyroid hormone-induced increases in GFR, as described in hypothyroid dogs (Gommeren et al. 2009) and humans (Villabona et al. 1999, den Hollander et al. 2005, Dousdampanis et al. 2014) and in normal cats (Adams et al. 1997a).

It might be logical to assume that diagnostic tests proven useful for identifying hypothyroid dogs would be equally useful in cats with iatrogenic hypothyroidism. However, canine and feline hypothyroid disease differ markedly in pathogenesis (immune-mediated thyroiditis or idiopathic atrophy in dogs versus iatrogenic ¹³¹I destruction of thyroid tissue in cats); time from onset to diagnosis (many months to years in dogs versus a few weeks to months in cats); and the pattern of serum T₄, fT₄, and TSH profiles (Ferguson 2007, Graham et al. 2007, Mooney 2011). For example, most dogs with hypothyroidism have low T₄ and fT₄ concentrations, whereas 46 and 75% of our hypothyroid cats maintained low-normal serum concentrations of T₄ and fT₄, respectively. The reason for the substantially higher proportion of normal T₄ and fT₄ values is unclear, but cats with iatrogenic hypothyroidism might simply have had a milder (partial) deficiency of thyroid hormone secretion than do most hypothyroid dogs.

While serum TSH concentration in cats shows high diagnostic accuracy for iatrogenic hypothyroidism, such accuracy is lower in dogs, with a reported diagnostic test sensitivity of only 58 to 87% (Peterson et al. 1997, Dixon & Mooney 1999, Boretti & Reusch 2004). In this regard, hypothyroid cats are similar to humans in which measurement of serum TSH is the most sensitive test for hypothyroidism (Landenson 2013).

Of interest is how hypothyroidism can develop clinically when serum T₄ and fT₄ concentrations remain within the low end of the reference interval. This is not just observed in cats (Lucy et al. 2017). The pattern of low-normal (within the lower end of the reference interval) T₄ or fT₄ concentration with high TSH concentrations (as seen in about half of our cats) is commonly recognised in human patients and referred to as subclinical (Cooper 2001, Vanderpump & Tunbridge 2002, Fatourechi 2009) or mild hypothyroidism (Aoki et al. 2007). Recent prevalence studies of human hypothyroid patients reveal that most (up to 95% in some reports) have subclinical (mild) hypothyroidism, with overt hypothyroidism being less common (Canaris et al. 2000, Hollowell et al. 2002, Aoki et al. 2007, Empson et al. 2007, Hennessey & Espaillet 2015).

In humans, measurement of TSH is much more sensitive than T₄ or fT₄, for detecting small alterations in thyroid function (a twofold change in fT₄ will produce a 100-fold change in TSH) (Spencer et al. 1990, Benhadi et al. 2010); TSH concentrations tend to rise outside the reference interval before T₄ or fT₄ fall to subnormal concentrations (Soldin 2013). The reason why some human patients can have “normal” T₄ and fT₄ within the reference interval and still show clinical signs of hypothyroidism can be explained, at least in part, by the fact that individual daily variation in serum T₄ and fT₄ concentrations is much narrower than the population-based reference interval (Nagayama et al. 1993, Andersen et al. 2002, Andersen et al. 2003). Such a narrow individual daily variation for total T₄ was also recently reported in clinically normal cats (Falkeno et al. 2016), which indicates that population-based reference intervals for T₄ might be of limited utility for diagnosis of feline hypothyroidism as well. Clinically, this means that a T₄ or fT₄ concentration within the reference interval might not actually be appropriate for that individual. In subclinical hypothyroidism, it is argued that if the serum TSH concentration is high, then T₄ and fT₄ are not truly normal for that individual, even if they remain within reference intervals (Cooper 2001, Vanderpump & Tunbridge 2002, Fatourechi 2009, Garg & Vanderpump 2013). Put differently, while higher-circulating TSH will increase thyroid hormone secretion, the additional T₄ and fT₄ does not fully compensate for the underlying deficiency in such cases. According to this view, subclinical hypothyroidism represents thyroid failure – i.e. less than full compensation for the diminished function of the thyroid gland (Medermott & Ridgway 2001, Ruggeri et al. 2011). Given the fact that cats might not show overt signs of hypothyroidism but do become azotaemic, the term subclinical hypothyroidism might not apply to many cats with iatrogenic hypothyroidism. Other terms, such as mild hypothyroidism, mild thyroid failure, partially compensated hypothyroidism or simply hyper-thyrotoxaemia with low-normal thyroid hormone concentrations, might better describe this syndrome in cats that develop azotaemic hypothyroidism after treatment for hyperthyroidism.

There are a number of other mechanisms that could be proposed to explain how clinical hypothyroidism (i.e. that manifested primarily by development of azotaemia) could develop when serum T₄ and fT₄ concentrations remain within the low end of the reference interval. For example, the kidney's response to falling thyroid hormone concentrations in treated hyperthyroid cats may differ from that in euthyroid cats that have lowered serum T₄ and fT₄ concentrations due to non-thyroidal illness – in other words, this may be a unique response that is more dependent on the interaction of the kidney and chronic thyroid excess followed by an abrupt fall in circulating thyroid hormone concentrations than a consequence of the actual concentrations of T₄ and fT₄ themselves. It is also important to realise that the TSH receptor is also expressed in kidney tissue (Williams 2011), and studies of human patients indicate a possible direct untoward effect of high TSH concentrations on kidney function, as reflected by reduced GFR (Sun et al. 2012, Tsuda et al. 2013). Therefore, a high serum TSH, even when serum thyroid hormone concentrations remain within the normal range, might directly suppress renal function in subclinical hypothyroidism.

One question that arises from this study is this: would serum TSH measurements be as useful in differentiating hypothyroid cats from euthyroid cats with no or lesser degrees of azotaemia? This study was not designed to address that question, but our data suggest that finding a clearly high TSH concentration (>0·9 ng/mL, i.e. thrice the upper limit of the TSH reference interval) in a cat treated with ¹³¹I is clearly consistent with iatrogenic
hypothyroidism. As lesser elevations in serum TSH concentra-
tions can be transient, with complete recovery of pituitary-thyroid
function in about a third of cats (Petersen & Rishniw 2016), we
would not recommend initiating levothyroxine replacement based
on a single high TSH measurement (or low T4 measurement)
alone, especially if the cat is not azotaemic. Continued monitor-
ing of the cat at three- to six-month intervals, along with serum TSH, T4 and fT4 and creatinine concentrations, is recommended.
If low serum T4 and high TSH concentrations persist, levothyrox-
ine is recommended, especially if a new or worsening moderate
azotaemia (≥220µmol/L) develops. Supplemental levothyroxine
replacement for non-azotaemic cats should also be considered if
serum TSH concentrations remain very high (>0.9 ng/mL) and if
other clinical signs (e.g. lethargy, hair loss) develop.

There are a number of limitations to this study. First, although we used quantitative thyroid scintigraphy to determine
thyroid status and separate 131I-treated cats into euthy-
roid and hypothyroid groups, thyroid scintigraphy has not
been validated as a test for iatrogenic hypothyroidism in cats.
However, use of quantitative scintigraphy has been reported as
an aid to the diagnosis of spontaneous hypothyroidism in dogs
(Balogh et al. 1998, Diaz Espineira et al. 2007, Shiel et al. 2012)
and cats (Blois et al. 2010) and is commonly used to deter-
mine the success of post-131I ablation in human patients (Jung
et al. 2015, Oxdemir et al. 2016). More studies are needed to
validate thyroid scintigraphy as a diagnostic test for iatrogenic
hypothyroidism in cats, especially in cats with milder degrees
of thyroid insufficiency and lesser elevations in serum TSH
concentrations. Nevertheless, the good agreement between
the scintigraphic diagnosis and TSH-based diagnosis suggests that
our use of scintigraphy was justified. Finally, more studies need
to be conducted with larger numbers of cats on the effect of
levothyroxine on the progression of CKD and survival time to
document the apparent beneficial effect of thyroid hormone
replacement in azotaemic, hypothyroid cats, especially those
cats with subclinical hypothyroidism in which the serum TSH
is high, but T4 and fT4 concentrations remain within the lower
limits of the reference interval.

Cats are considered resistant to developing clinical signs of iat-
rogenic hypothyroidism, and published reports suggest that most
hypothyroid cats do not require thyroid hormone replacement.
Other than weight gain and decreased activity – both of which
could be attributed to successful treatment of hyperthyroidism
– we noted no other obvious findings of hypothyroidism. The
most common clinical sign reported in our 42 azotaemic cats
was polyuria and polydipsia, with a similar prevalence in both
euthyroid and hypothyroid cats. Why more cats do not develop
more overt signs of hypothyroidism is unknown, but it is possible
that the development of overt clinical signs might take longer
than the observation period in our study. Additionally, as noted
above, many hypothyroid cats likely fail to develop severe clin-
cal signs because of the mild nature of their disease. The only
clinical implication or concern for why we need to diagnose and
treat most of these cats is the unmasking of azotaemic CKD and
the fact that levothyroxine supplementation will lessen their
azotaemia. Therefore, even if there are minimal clinical signs
associated with iatrogenic hypothyroidism in most cats, diagnos-
sis and treatment of 131I-treated hypothyroid cats that develop
azotaemic CKD are appropriate to protect kidney function and
hopefully improve survival.

Acknowledgments
We thank Carol Castellano for technical assistance as well as edit-
ning and critical reading of the manuscript.

Conflict of interest
Dr. Rhett Nichols serves as an internal medicine consultant for
Antech Diagnostics.

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