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Relating Tumor Score to Hematology in Green Turtles with Fibropapillomatosis in Hawaii

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ABSTRACT: The relationship between hematologic status and severity of tumor affliction in green turtles (*Chelonia mydas*) with fibropapillomatosis (FP) was examined. During 1 wk periods in July 1997 and July 1998, we bled 108 free-ranging green turtles from Pala'au (Molokai, Hawaii, USA) where FP is endemic. Blood was analyzed for hematocrit, estimated total solids, total white blood cell (WBC) count and differential WBC count. Each turtle was assigned a subjective tumor score ranging from 0 (no visible external tumors) to 3 (heavily tumored) that indicated the severity of FP. There was a progressive increase in monocytes and a decrease in all other hematologic parameters except heterophils and total numbers of white blood cells as tumor score increased. These data indicate that tumor score can relate to physiologic status of green turtles afflicted with FP, and that tumor score is a useful field monitor of severity of FP in this species.

Key words: *Chelonia mydas*, fibropapillomatosis, field monitoring techniques, green turtle, hematology.

Green turtles (*Chelonia mydas*) are herbivorous marine reptiles frequenting pastures of algae and seagrasses in tropical and subtropical coastal areas throughout the world. Green turtles are considered endangered worldwide, and a discrete population exists in the Hawaiian islands (Bowen et al., 1992).

Fibropapillomatosis (FP) of marine turtles is a debilitating neoplastic disease with a global distribution (Herbst, 1994) that was originally described in green turtles from the Florida Keys (USA) (Smith and Coates, 1938). In Hawaii (USA), FP was first documented in 1958, and prevalence in certain coastal habitats has increased or remained high since systematic surveys were started in the early 1980's (Balazs, 1991).

Clinical pathology studies by Adnyana et al. (1997) and Aguirre et al. (1995) showed that green turtles afflicted with FP were anemic and hypoproteinemic. In addition, green turtles with FP were leukopenic, lymphopenic, eosinopenic, heterophilic, and neutrophilic compared to tumor-free animals (Aguirre et al., 1995). However, a recent characterization of white blood cells from green turtles in Hawaii has shown that this species lacks neutrophils (Work et al., 1998).

A subjective index to assess the degree of severity of external FP in green turtles in Hawaii was devised by Balazs (1991) and has been used in Hawaii to categorize the severity of the disease in free-ranging turtles. The purpose of the present study was to relate tumor score to hematology of green turtles using recently developed criteria to classify green turtle white blood cells (Work et al., 1998).

The study site was the Pala'au foraging pasture, where FP is endemic, along the southwest coast of the island of Molokai (Hawaii; 21°10′N; 157°45′W). Turtles were harmlessly captured by hand after they swam into a maze-like arrangement of small-mesh nets set on a shallow reef flat. The turtles were unable to exit but did not become entangled. After processing and release, turtles behaved normally and swam away with no difficulty.

For each turtle, straight carapace length was measured with calipers, but gender was not determined. Individual tumors were classified on the basis of four approximate sizes: less than 1 cm (Size A); 1 to 4 cm (Size B); greater than 4 to 10 cm (Size C) and greater than 10 cm diameter (Size D). Turtles were then assigned an overall tumor score ranging from 0 to 3 based on the numbers of tumors within

TABLE 1. Numbers of each size class of tumors used for placement into a particular tumor score category for green turtles afflicted with fibropapillomatosis in Hawaii.[a]

	Tumor score			
	0	1	2	3
Tumor size				
(A) <1 cm	0	1–5	>5	>5
(B) 1–4 cm	0	1–5	>5	>5
(C) >4–10 cm	0	0	1–3	>4
(D) >10 cm	0	0	0	>1

[a] Based on the actual count of tumors on 108 turtles from Pala'au Molokai subjectively scored in this study.

tumor size-class (Table 1). The tumor score reflected the spectrum of severity of FP in green turtles ranging from non-afflicted (0) to lightly (1), moderately (2), and heavily (3) afflicted (Balazs, 1991).

Animals were bled (10 cc) from the cervical sinus (Owen and Ruiz, 1980) with a sterile syringe and 0.9 × 38 mm needle. Blood was processed for hematocrit, estimated total solids, total white blood cell (WBC) and differential WBC count (Work et al., 1998). In all, we bled 54, 24, 18 and 12 animals that were assigned a tumor score of 0, 1, 2, and 3, respectively, during 1 wk periods in July 1997 and July 1998.

Hematologic parameters were compared between the tumor score groups using the pooled data of turtles from 1997–98. We used one-way ANOVA or Kruskall Wallis test depending on whether data fit the assumptions of normality and equal variance (Daniel, 1987). Post-hoc comparisons were made using Student-Newman-Keuls test or Dunn's test for significant differences with ANOVA or Kruskall Wallis tests, respectively. To maintain an experiment-wide error rate of <0.05, we used a Bonferroni adjustment (alpha/n) where n was the number of analytes being compared (Rice, 1989). Sigmastat (SPSS, Chicago, Illinois, USA) was used for all statistical comparisons.

Straight carapace length ranged from 32.6–76.9 cm and did not differ significantly between tumor score categories (Table 2). There was a progressive decrease in hematocrit, estimated total solids, lymphocytes, basophils, eosinophils, and total white blood cells and a progressive increase in heterophils with increasing tumor score. Monocytes also progressively increased with tumor score except in turtles with a tumor score of 1. Significant differences were seen between tumor scores for hematocrit, estimated total solids, and eosinophils.

Scoring severity of fibropapillomatosis in green turtles has been done by Wood and Wood (1993) in the Cayman Islands. However, our system takes into account both size and numbers of tumors whereas Wood and Wood (1993) only used tumor size to assess a tumor score. Attempts to relate tumor scores to physiologic indices

TABLE 2. Hematology of green turtles from Pala'au (Molokai, Hawaii, USA) partitioned by fibropapilloma tumor score (numbers in each score category are mean and standard error).

	Tumor score			
	0 (n = 54)	1 (n = 24)	2 (n = 18)	3 (n = 12)
Straight carapace length (cm)	51.9 ± 1.3	56.2 ± 1.9	54.2 ± 2.2	56.1 ± 2.4
Hematocrit (%)	38 ± 1a	36 ± 1b	33 ± 1b	24 ± 1c
Estimated total solids (g/dL)	4.0 ± 0.1a	3.6 ± 0.1b	3.4 ± 0.2b	2.5 ± 0.3c
Lymphocyte ($10^3/\mu L$)	8.21 ± 0.65	7.05 ± 0.61	6.50 ± 1.00	5.61 ± 1.15
Heterophil ($10^3/\mu L$)	1.26 ± 0.09	1.41 ± 0.12	1.42 ± 0.16	1.91 ± 0.31
Monocyte ($10^3/\mu L$)	1.08 ± 0.08	0.96 ± 0.08	1.21 ± 0.19	1.45 ± 0.33
Eosinophil ($10^3/\mu L$)	1.25 ± 0.10a	1.09 ± 0.17ab	1.06 ± 0.16ab	0.50 ± 0.13b
Basophil ($10^3/\mu L$)	0.08 ± 0.02	0.08 ± 0.04	0.06 ± 0.02	0.05 ± 0.02
Total White cell count ($10^3/\mu L$)	11.89 ± 0.80	10.59 ± 0.85	10.24 ± 1.22	9.52 ± 1.67

a,b,c-values within a row are significantly different from each other ($\alpha < 0.006$).

are rare. For example, Balazs et al. (1998) found that growth rates of turtles decrease with increasing tumor score. The results of the present study reveal that the subjective tumor score is reflective of the hematologic status of green turtles afflicted with FP. Our results confirm those of other studies which showed that green turtles with FP are anemic (Aguirre et al., 1995; Adnyana et al, 1997) and hypoproteinemic (Aguirre et al., 1995). Our findings of elevated lymphocyte, and heterophil counts, and decreased white cell counts in turtles with FP also agreed with those of Aguirre et al. (1995). Aguirre et al. (1995) did not report categorization of turtles according to tumor score, so comparisons with their findings are incomplete.

It is likely that most of the turtles in this study were infected with vascular trematodes (Dailey and Morris, 1995; Graczyk et al. 1995; Aguirre et al., 1998; Work and Balazs, 1998). The effect of these parasites on green turtle hematology is unclear. Adnyana et al. (1997) postulated that anemia in turtles with FP was due to vascular trematode infection, while Aguirre et al. (1995) postulated chronic disease (FP) as a cause of the anemia. On histology, turtles respond to vascular trematodes with lymphocytes or eosinophils (Glazebrook et al., 1981; Work and Balazs, 1998; Aguirre et al., 1998). Assuming that all green turtles with FP have heavier vascular trematode intensities (Adnyana et al., 1997), sequestration of eosinophils in tissues as a result of inflammatory response to trematodes may explain eosinopenia and lymphopenia observed here.

Chronic inflammation was probably responsible for the progressive monocytosis with increasing tumor score (Campbell, 1996). Based on elevated cortisol levels, Aguirre et al. (1995) attributed heterophilia and lymphopenia in green turtles afflicted with FP to stress and immunosuppression. Although tumor score was not significantly related to carapace length, there was undoubtedly some sex and age variation in the animals we sampled, and this may account in part for the inconsistent pattern for some blood analytes. Frair (1977) and Frair and Shah (1982) showed that hematocrit and total protein varied linearly with carapace length. These confounding factors could be eliminated by sampling animals in each tumor score for fixed sets of carapace lengths. However, this would be achievable only over a prolonged time span and may introduce confounding temporal factors, such as season, which can affect hematologic parameters in reptiles (Duguy, 1970). It is unlikely that season played a role in the present study since animals were sampled within a one week period during the same season albeit during two different years. Presently, determining gender of immature marine turtles can only be done by laparoscopy or hormonal assays making sampling and segregation by gender in the field difficult and not always practical.

Tumor scores underline the importance of quantifying severity of disease in animal populations. With appropriate refinement of this and other demographic methods, tumor score may serve as a predictor of whether turtles are likely to survive or succumb to fibropapillomatosis. Other research might focus on the cause of anemia and hypoproteinemia in turtles with tumors, assessing whether animals with lymphopenia are immunosuppressed, and if vascular trematode infection impacts hematology.

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